Chapter 5

Discussion

1. The concentration of potassium chlorate on flower induction of longan

Logan plants treated with KClO₃ at the concentration of 0.05 and 0.10 g/pot (50 and 100 ppm) were flowering earlier (29 and 28 days after treatment) than at 0.15 g/pot (42 days) which had shown smaller and shorter inflorescences. Moreover, the percentage of flowering at the lower concentrations of KClO3 was 100 %, whereas 52.78 % found at KClO3 0.15 g/pot. Higher concentration of KClO₃, 1-12 g/pot caused leaves chlorosis within 3 days, thereby leaf blast or fallen leaves were observed. All of these plants had not flowering. It may be due to high concentration, 0.5 mol/m3 of chlorate toxic to most plants (Siddigi et al. 1992). The similar results were found for longan in South Florida. The 22.1 m² average canopy width of longan were applied with KClO₃; 0, 114, 227 and 340 g per plant found that the percentage of terminals flowering was greater for plant treated with 114 g and 227 g (92% and 91% respectively) than 340 g (84%). The percentage of terminals that set fruit was also greater for plants treated with 114 g and 227 g (88% and 83% respectively) than 340 g (79%), but fruit development appeared to be normal (Crane, 2003). Apart from toxicity of KClO₃, no full flowering may be due to some signal from leaves. In cultured maize apices, immature leaves are required to determine the developmental potential of the apex. Leaving the 4 to 6 youngest leaf primodia on the excised apices prevents the resetting of the development program, indicating that some signal from the leaves influences development of the apex. Because excised apices revert to producing a full set of leaves before they produce flowers (Levy and Dean, 1998).

Sex expression is affected by plant hormones in many monoecious and dioecious species. Environmental factors, such as temperature and day length can trigger the reversion of sex in several species, possibly caused by changes in hormone levels (Lebel-Hardenack and Grant, 1997). Longan and litchi produce staminate flowers (pistil non-function), pistillate flowers (stamen non-function) and hermaphrodite flowers. These flowers are initiated from a

bisexual primodium and then develop into androecium and gynoecium. The ratio of female flower was 5-20 % in litchi and 10-30 % in longan. It is several weeks from floral bud initiation to the sex differentiation, providing a critical period in flowering and fruit set (Zheng *et al.*, 2001). The number of female flower at 0.05 g KClO₃/pot was 3 times higher than at 0.15 g/pot, may caused by changing in internal hormones that involved with sex expression of flower and fruit set. The studied from sugar cane, rice and maize suggested that non-13-hydroxylated GAs, such as GA_4 might be involved in sex differentiation and particularly in the development of male reproductive organs (Hooykaas *et al.*, 1999). In longan, content of IAA was high during staminate differentiation and low during pistillate differentiation, whereas ABA increased at beginning of pistillate differentiation, but remained low in the staminate flower until anthesis. A high ratio of IAA + zeatin + GA_{1+3} : ABA was associated with staminate differentiation while the reverse was associated with pistillate differentiation (Lin *et al.*, 2001).

When the experiment 4 was conducted in March and April, the temperature was high, so the flower buds were observed 18 days after treated with KClO₃, which was about 11 days earlier than the first experiment. The flower bud formation was identified by microscope 16 days after treatments. In tobacco, analysis of the data indicated that a meristem also changed from a vegetative state to a florally determine state in about two days. It appears that a tobacco meristem initiates floral morphogenesis after it has become florally determined without additional developmental signals (McDaniel, 1996).

2. Root growth and development in hydroponics

Root growth and development in hydroponics seems not affected by KClO₃. Root growth may compete with canopy vegetative and reproductive growth for carbohydrates. A distinct flushing pattern of root growth that alternated with stem growth was reported for avocado and *Citrus sinensis*. However, a continuous root growth cycle that appears independent of the timing of stem growth cycle was reported in mango and *Citrus aurantium*. The maximum root extension rate for six replications was 7.4 mmday⁻¹. Most young litchi root tips that was observed never ceased growth throughout the period of which they remain visible on the plane of observation window (Marler and Willis, 1996). Root growth of longan may be has a similar pattern to litchi for they are belonging to the same Family, Sapindaceae. On the other hand, study

about root growth in hydroponics with higher concentration of KClO₃ may be cannot explain for root growth in fine sand. As longan is not tolerance to flooding then hydroponics is the limiting factor for growth and developing. In tomato that cultured in a flow-through hydroponics had a significant decline in respiration capacity was observed for roots grown in small containers after 18 days of cultured. It was concluded that a decline in root respiration capacity represents a significant indication of reduced root metabolism (Peterson et al., 1991).

3. Root respiration

Respiration is usually rapid in tissues with high energy demands, the rapidly growing tissues, such as the elongation zone of roots. Plant respiration can also increase rapidly in response to both biotic and non biotic stress (Millar *et al.*, 1998). Respiration of roots in this experiment was not significant difference may be because of very low concentration of KClO₃, 0.05 mg/pot did not harm the plant root, since root electrolyte leakage and root growth were not difference. The rate of root respiration may affect by other factors. Root respiration declined as root diameter increased and was lower at deeper soil depths than at the soil surface. Respiration rates for roots < 0.5 mm in diameter were 2.4 to 3.4 times higher than those of root in larger diameter classes. Low respiration rates consistent with structural and transport functions rather than with active nutrient uptake and assimilation (Preqitzer *et al.*, 1998).

4. Photosynthetic rate and stomatal conductance

Photosynthetic rate and stomatal conductance were high at 8.00 a.m. but declined from 10 a.m. to 4 p.m. in most plants. The photosynthetic rate was hardly detected in the afternoon, where negative values of photosynthetic rate were observed. Fukamachi *et al.* (1998) also found that net assimilation rates decreased in longans and mangoes as temperature increased, but the decline was greater in longans than in mangoes. As temperature increased, stomatal conductance decreased and intercellular CO₂ concentration increased for both species, especially longans. A significant positive correlation between stomatal conductance and net assimilation rate were found in longan at 30 and 33°C, but not at 36°C where the photosynthetic rate was very low. In apple, also found a weakness of the sub-model for modeling stomatal regulation of apple tree and the climatic data measurements (Costes *et al.*, 2002). Although, photosynthetic rates of treated

and untreated with KClO₃ of longan plants were the same, but synthesis of sucrose and starch may be difference. Laporte (1998) studied in transformed tomato plants found that sucrose phosphate synthase (SPS) transformed plants had higher rates of sucrose synthesis than wild-type plants. Starch synthetic rates were lower in the transformed plants so there was no change in net photosynthesis. The SPS-transformed plants started to flower earlier, had increased shoot biomass and decreased root biomass, and grew faster than wild- type plants. In this experiment, shoot TNC and RS of treated plants were higher than untreated plants. Therefore, less starch production or less in carbohydrate metabolism of ceased growing shoot may cause by higher TNC and RS of shoot rather than higher photosynthesis in plants treated with potassium chlorate.

Stomatal behavior could not be detected, as longan stomata are very small and sunken. However, stomata responses to water availability in the soil, water decreases, stomatal conductance also decrease at any particular level of evaporative demand. Leaf-specific hydraulic conductance (K_L) and the threshold of leaf water potential can explain the variation among the species in stomatal response to soil and atmospheric water deficits (Bond and Kavanagh, 1999). Leaf water potential, stomatal conductance and assimilation were directly proportional to K_L ($R^2 > 0.90$), indicating that changes in K_L may affect plant carbon gain (Hubbard *et al.*, 2001). Therefore, when soil moisture decreased in the afternoon the stomatal behavior has been changed to partially closed, thereby brought about the decreasing of stomatal conductance and photosynthetic rate.

5. Electrolyte leakage and peroxidase activity

Electrolyte leakage, EL, is an indicator of cell injury, the measuring was developed to predict freezing tolerance of leaves and roots of many kind of plants. Cell killed by freezing temperatures leak electrolytes and other cell contents as a result of membrane disruption (Maier et al, 1994). In this experiment, EL in both roots and leaves in treated plants are not significant difference from untreated plants. It may be because low concentration of KClO₃ did not damage to roots and leaves tissue. Peroxidase activity was also detected to prove the assumption that plants increase its activity when subject to KClO₃. The results found that the peroxidase activity of roots and leaves of treated and untreated plants did not significant difference. Plant has the protection system when it was invaded or suffered by diseases, toxic chemicals or stress

environment (Wititsuwannakul et al., 1997). The super-oxidized anion radicals (O_2) produced by chlorophyll under light, the H_2O_2 and hydroxyl radical (OH) formed from the dismutation of O_2 , are known as active oxygen. Active oxygen directly or indirectly activates the peroxidation of membrane lipids, damaging membrane. There are two systems, enzymatic (superoxide dismutase, peroxidase and catalase) and non enzymatic (antioxidants) system, which eliminate active oxygen in plants. In enzymatic system, superoxide dismutase is the most important enzyme and catalyzes O_2 into H_2O_2 . Similarly, peroxidase and catalase convert H_2O_2 into nontoxic water. The decreasing of these enzymes reduced the content of chlorophyll of leaves. Under normal conditions, the amount of active oxygen is balanced by elimination (Xu et al., 2001). However, peroxidase activity of leaves of treated plants at the fourth week of this experiment was significantly greater than untreated plants. The study in Capsicum annuum leaves showed that floral development is accompanied by a significant increase in the level of soluble leaf peroxidase, independently of leaf position along the internodes and therefore independently of the leaf age. The increase in peroxidase activity is due to a general increase in the activity of all the preexisting peroxidase isoenzymes (Bernal et al., 1993).

6. Changes of some essential substances and mineral nutrients

6.1 Carbohydrate, nitrogen, phosphorus and potassium

Chlorophyll a, chlorophyll b and total chlorophyll contents between treated and untreated plants were not significant difference. Millar et al. (1998) suggested that the content of chlorophyll declined significantly only under N-deficiency alone. In this studied, total nitrogen of leaves of treated plants was not differing from untreated plants. Moreover, it was also reported that chlorophyll of longan leaves of plants treated with KClO₃ and placed under variation of light intensity was not difference. However, chlorophyll degradation of detached leaves were significant difference on the first week of treatment. The chlorophyll content of treated plants was lower than untreated plants on the third day. Katz et al. (1978) reported that salinity might enhance leaf senescence, indicated by inhibition of protein synthesis and more rapid chlorophyll degradation. Kinetin partially counteracts these effects.

Root TNC at the first week of plants treated with KClO₃ were significantly higher than untreated plants, but root RS was significantly lower than untreated plants. However, root TNC

on the third and fourth week declined while it was increasing in shoots. It may be like Chrysanthemum, photoperiodic induction of the flowering stimulus shoot carbohydrate rather than direct competition for resources between flowers and developing roots (DeVier and Geneve, 1997). Shoot TNC and RS of treated plants were significantly higher on the second to the fourth week of treatments. Similar to S. alba that sucrose increased precedes the increase cell division (Bernier, 1993). Hexose metabolism is associated with meristematic activity (cell division) in the developing embryo, whereas sucrose metabolism is associated with starch and protein storage function (Smeeken, 2000). It seems like there was some activity in shoot on the second week. However, Kinet (1991) suggested that the increased sucrose level in phloem sap of Xanthium and Sinapsis indicated that it did not result from a higher demand of apical meristematic tissues, which are activated later. The enhanced supply of sucrose to the apex could thus act as a message-like factor triggering some essential events of the flowering process at the meristem. Leaves TNC was about 50% lower than root and shoot TNC. It may due to photosynthate of leaves was exported to roots and shoots so only a few was left. Therefore, no difference between leaves TNC of treated and untreated plants had been observed. Leaves may sometimes have a high concentration of starch, but because of their small weight, their contribution to the total pool of reserves is small compared with the trunk and roots (Menzel et al., 1995). In addition, increased carbohydrate levels of leaves lead to inhibition of photosynthesis and a decrease in ribulose-1, 5- bisphosphate carboxylase protein, other Calvin cycle enzymes and chlorophyll (Smeekens, 2000). However, leaves RS of treated plants at the fourth week of treatments was significantly lower than untreated plants. It may due to RS was provided for sucrose formation for exporting to the inflorescences. In lychee, carbohydrates have been implicated in floral initiation but evidence for this is not clear, however, there was a strong correlation between flowering and starch leaves under different temperature (Menzel et al., 1989). Potassium chlorate more or less induced changing in root and shoot TNC and RS involving flower induction in longan.

There was no effect of KClO₃ on TN of roots, leaves and shoots, except shoot TN on the fourth week of treatments. Shoot TN of treated plants at the fourth week was highly significant difference than untreated plants. Nitrogen is a component of protein, membrane, nucleic acid and secondary metabolites, so it is essential for plant growth and development. Similar to shoot TN,

nitrate content of shoot on the fourth week was also high in treated plants. Nitrate is reduced to nitrite and ammonium, a component of amino acid necessary for inflorescence development. Root NO₃ of treated plants at the first three weeks seem to be higher but only NO₃ content in the second week was significantly difference compared with untreated plants. Root TN was not significant, higher NO₃ of root may be due to the decreasing of NO₃ reduction. Chlorite, a reduced form of chlorate may inactivate NR activity (LaBrie, 1991), or maybe because of high auxin content of root. Auxins are known to induce H⁺ extrusion from plant tissues and acidification in the cell. Increased in H⁺ concentration results in increased glutamate dehydrogenase (for glutamate formation) and decreases glutamine synthetase (Sahulka, 1981). In *Arabidopsis*, demonstrated that the four amino acid, glutamine, glutamate, aspartate and asparagine can account for 60-64 % of the total free acid present in the leaves and transported in the vascular tissue (Lam et al., 1996). Therefore, if the ratio of certain amino acids have been changed, thereby new proteins might be induced for the other activities. Moreover, study on *Lemna paucicostata* suggested that free lysine of plants increased during N-free culture and lysine had a flower- inducing effect on the plant (Tanaka et al., 1993).

C: N ratio of shoots was higher in treated plant at the second week of treatment just before flower bud initiation. Smeeken (2000) indicated that carbon and nitrogen are tightly linked and their interactive signal may be controlled by phytohormones. In *Arabidopsis*, carbohydrate and cytokinins are two components of the complex floral stimulus. Amino acids could contribute another components of this floral signal and that an imbalance in the C and N availability at the apex may be critical for the floral transition to occur (Corresier, 1998). After flower buds were induced, shoot C: N of treated plants was lower on the fourth week, it may because shoot needs to consume more nitrogen for cell activity.

The percentage of phosphorus of shoots was a little bit higher than leaves and roots. However, phosphorus in treated and untreated plants was not difference in roots, leaves and shoots. It may because phosphorus of some systems in plant is equilibrium. The transport of both inorganic and organic phosphates across the membrane is brought about by phosphate translocator, which couples the uptake of inorganic phosphate with the export of triose phosphates. Not only phosphate taken up by root is supplied to young leaves, but also with phosphorus originating from the older leaves (Mengel and Kirkby, 1987).

The percentage of K in shoot of treated plants at the fourth week was significantly higher than untreated plants may be due to potassium is an important factor for loading and unloading. However, Menzel, et al. (1989) suggested that variations in nutrient concentration could not account for difference in vegetative growth and flowering. The concentration of most nutrients in the shoots of litchi cv. TaiSo was generally increased by conditions that favored uptake and reduced by improper conditions.

6.2 Plant hormones

Auxin like substances of treated plants at the first and second weeks were higher than untreated plants, similar to IAA in primary root of *Euphobia escular* which was high, but decreased by 40% after flowering (Nissen and Foley, 1987). On the other hand, shoot auxin tends to decrease in tobacco bud before flowering (Bernier *et al.*, 1993). Hegele *et al.* (2003) also found that IAA content of shoot was a little bit lower on the first week of longan treated by KClO₃.

Cytokinin-like substances of root of treated plants at the second week were higher than untreated plants. Similar to S. alba which the levels of cytokinins of root exudate elevated before flowering are apparently due to activation of cytokinin release rather than biosynthesis. The major cytokinin in root exudate is zeatin riboside and a minor component is isopentenyladenine (Bernier et al., 1993). Study in wheat, Kudoyarova et al. (1999) found that growth rate of second leaf of seedlings decreased within 15 min. after cooling of roots at 4 °C. Cooling induced a transient increase in root cytokinin content, followed by a more than 3- fold decrease. Cytokinin concentration in xylem sap of Rosa hybrida also decreased as bud break occurred (Dieleman et al., 1997). The transient increase was attributed to decrease xylem transport from roots to shoots after cooling, after which the decrease is caused by inhibition of cytokinin biosynthesis of roots, leading to decrease growth of the shoot. It is may be one of the reasons that the shoot stop growing before flower bud initiation. During floral initiation in longan shoot, a large decrease in cytokinin glucosides and an increase in zeatin and zeatin riboside activities in buds were found which was higher than cytokinin content at the stage of leaf flush (Chen et al., 1997). The observation in tobacco supports the idea that enhanced cytokinin levels are essential for cell differentiation and organogenesis, but not for floral evocation. This seems to be substantiated by the observation during the prefloral phase, no organogenesis take place, a phenomenon that

eventually may prove to be provoked by the significantly reduced cytokinin levels (Dewitte et al., 1999). Study on tobacco, found the agreement with the hypothesis that the cell competence for cytokinin autonomy associated with increased endogenous cytokinin levels and that the maintenance of this autonomy is based on the positive feedback when cytokinin induce their own accumulation or inhibit their own degradation. It was reported that induced or enhanced free cytokinin accumulation after partial or total auxin deprivation or inactivation of auxin synthesizing genes found in transformed plants (Hooykaas et al., 1999). Therefore, ratio of auxin and cytokinin content may be involved to plant growth and development.

Gibberellin in roots was increasing gradually from the first week of both treated and untreated plants with no significant difference. It may be because GA contributes root growth rather than involves in signal to flower induction of longan. GA biosynthesis occurs mainly in actively growing tissue and declines as growth ceases (Hooykaas et al., 1999). In pea, GAs are important for normal root elongation. It was found that, dwarf mutants of pea with impaired GA biosynthesis reduced GA levels in root tips and tap root elongation (Yaxley et al., 2001). Moreover, GA generally inhibit flowering of woody plants (Blazquez et al., 1998).

Ethylene in roots was increasing like GA after treatment, however ethylene in treated plants was significantly higher from the second week. High root auxin may be induce 1-aminocyclopropane-1-carboxylic acid synthase, a protein essential to ethylene formation, but the protein is rapidly inactivated with an apparent half-life of 35 min in pea and 30-100 min in tomato. In contrast, study in *Chenopodium rubrum* indicated that root ethylene showed no response to IAA or darkness. Nevertheless, the period of highest sensitivity to flowering coincided with peak ethylene production in 4-6 day- old plants (Ullmann *et al.*, 1985). However, one characteristic features of ethylene production of higher plants is that the rate of production frequently changes not only from environment stimuli but also during normal development (Hooykaas *et al.*, 1999). For example, ethylene production in tomato appeared at a time when adventitious roots were initiated (Peterson *et al.*, 1991) and ethylene production of shoot and leaf may be signaled by root. Roberts *et al.* (2002) study on *Lycopersicum esculentum* grown in compacted soil, the observation found that the elevated ethylene production of plants was a consequence of enhanced conversion rather than elevated synthesis of ACC in the leaf tissue. It

was suggested that ethylene in leaf may be regulated by a root-source signal capable controlling ethylene production.

7. Correlation between chemicals

Correlation between TNC, RS and TN of roots, leaves and shoot of untreated plants were difference from treated plants. Once there was high level of N in roots, leaves and shoots, then low level of RS and TNC in shoots were found of untreated plants. In contrast, higher level of N, RS and TNC of shoots were observed in treated plants whereas N, RS and TNC of roots and leaves were low. Since high N needs for amino acid, nucleic acid and some secondary metabolite production, carbohydrate is provided as carbon skeleton for forming organic compounds for growth and development of new tissues and organs.

The correlation between N, P and K content of roots, leaves and shoots of untreated and treated plants were also difference. Nitrogen and K content had high positive correlation in roots and in shoots, but not in leaves of treated plants which high content of K, but low content of N was found. High content of K of leaves may activate ATPase which involved in loading and transport of sucrose. Potassium can also facilitate sucrose loading indirectly by increasing sucrose concentration in the apoplast of the leaf tissue, K⁺-sucrose cotransport at the plasma membrane of leaf cell. Potassium can contribute substantially to the total osmotic potential in the sieve tubes and thus to the volume flow rate. In plants well supplied with K⁺, the concentration of K and osmotic potential of the phloem sap and particularly the volume flow rate are all higher than in plants supplied with a lower level of K (Marschner, 1986). The function of P as a constituent of macro molecular structures is most prominent in nucleic acids. It is high in meristems and low in storage tissues. The amount of phospholipids and RNA synthesized is high but they are much more stable compounds and have a relatively low rate of synthesis. The actively metabolizing cell, energy-rich phosphates are characterized by extremely high rates of turnover, but high rate of synthesis. Therefore, a small amount of ATP can satisfy the energy requirement of plant cells (Marschner, 1986). It may be one of the reasons that why P content in plants has not difference between treated and untreated plants.

Higher concentration of IAA, cytokinin and ethylene in root were noticed at the second week, just before flower buds were visible. However, effect of hormones were less on root

growth and development, since no significantly increase of TNC and RS of root of treated plants were observed on the second to the fourth week. Moreover, root respiration was not difference. In contrast, TNC and RS were happened to increase significantly in shoot from the second to the fourth week. Therefore, change in root hormones more or less signal or effect the change of hormones in shoot for induces flowering of longan.

8. Compare to natural condition

Under natural condition, low temperature and drought, longan and litchi are induced to flower. The experiment in litchi suggested that day shoot temperatures and root temperatures interact to control the level of flowering. Whereas water stress appears to act by synchronizing vegetative dormancy in the branches before exposure to low temperatures (Menzel et al., 1995). Both kinds of stress involve a cessation of root growth, pointing towards a common hormonal mechanism. In Phalaenopsis subjected to low temperature (25/20 °C day/night), zeatin, zeatin riboside and dihydrozeatin levels in the leaves were higher than in the leaves exposed to high temperature treatments (ChinChih et al., 2000). In litchi at low temperature also found that shoot cytokinin was reached maximum content from two weeks prior to flowering until flowering (Naphrom et al. 2001). However, in longan 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). Plants under water stress, enhanced unloading in primary roots than in young leaves due to a stronger induction of vacuolar acid invertase activity in roots (Kim et al., 2000). Water stress also markedly increased in ethylene in root of papaya (Cruz et al., 2000). These phenomena are similar to longan plants subjected to KClO₃. Therefore, plant hormones and carbohydrate in roots may be part of inducing substances to promote flower initiation.

In this experiment, potassium chlorate did effect the content of carbohydrate, nitrate, auxin, cytokinin and ethylene in root. The changes in content of these components of roots correlated with the change in content of the components of leaves and shoots that regulate the flowering in longan. Potassium chlorate caused accumulation of nitrate in roots may be by lower nitrate reductase activity. The situation just like tobacco subjected to nitrogen starvation which roots showed a greater increase in sugar levels especially sucrose and fructose (Lancien et al.,1999). Starch may be accumulated in roots since there was no significant difference of root

fresh weight (data not shown). Interactions between ClO, and NO, transport were complex; 50 micromolar NO₃ acted as a mixed inhibitor of net ClO₃ uptake, but 50 micromolar ClO₃ had no significant effect on net NO3 uptake, and 500 micromolar ClO3 had no significant effect on 15 micromolar of NO₃ influx (Kosola and Bloom, 1996). However, chlorate toxic effects unrelated to nitrate assimilation and interactions between absorbed nitrate and chlorate (MacKown et al., 1996). The toxic and mutagenic effects of chlorate were not found when cells were grown either in darkness or in the presence of ammonium, conditions under which nitrate uptake is drastically inhibited. In addition, chlorate appears to be a mutagen capable of inducing a wide range of mutations unrelated to the nitrate assimilation pathway (Prieto and Fernandez, 1993). If chlorate can be mutagen, therefore, protein production in plants has been changed. These proteins might activate the flower-regulated genes. Chlorate may directly signal flowering genes or signals other chemicals such as hormones to induce flowering in longan. Chlorate and chlorite caused increasing root IAA (auxin), CK (cytokinin) and C₂H₄ (ethylene) that may induce changes in shoot hormones by decreasing level of IAA and CK but increasing level of C2H4. Chlorate also lower the level of nitrogen assimilation and higher the C:N ratio of shoot. Shoot C: N and growth checked might induce changes in shoot hormones, which activate the floral meristem identity genes and floral organ identity genes afterward.

Pathway of flowering may have many possible ways. However, as far as the data have been presented, it is not enough information to create a good model for flower induction of longan affected by potassium chlorate. One way to approach to the floral induction process is to isolate the genes identified by mutations and to study their properties. Once the gene is cloned, the encoded protein is purified to study its biochemical properties and to produce specific antibodies. The antibodies can be used to determine the tissues in which the protein is produced, as well as its localization within the cell. The localization may provide clues to the function of the protein. A protein in the shoot apex may be involved in the reception of flowering signals, whereas a protein presenting in the leaves may be involved in the production of flowering signals. The *constans* mutants of *Arabidopsis* are insensitive to day length and flower later than the wild type under long-day conditions. The wild-type *CONSTANS* gene is expressed in leaves, and the level of *CONSTANS* m RNA increases during inductive photoperiods (Zeiger, 1998).