

Chapter 2

Literature Review

2.1 Origin and distribution of *C. alismatifolia*

The genus *Curcuma* originated in the Indo Malayan region, which is comprised over 80 species of rhizomatous annual or perennial herbaceous (Purseglove, 1974), and is covered with widespread adaptation in diversified tropical condition from high altitude about 1,500 – 2,000 m above sea levels in Western Ghats and Himalayas (Larsen and Larsen, 2006). The center of diversity of the genus *Curcuma* is in the continental monsoon in Asia and the distribution is throughout tropical Asia from Western India to South Asia, South China, Papua New Guinea and finally to Northern Australia (Sirirungsa *et al.*, 2001). Especially Thailand, which locates in South East Asia, seems to be one of the most abundant diversity areas for *Curcuma*. About 50 species of the genus have been naturally recovered in Thailand (Larsen and Larsen, 2006; Sirirungsa *et al.*, 2001). *Curcuma* yields are mostly used for spice, dyes, perfumes, medicines and ornamental flowers (Haywood *et al.*, 1985).

C. alismatifolia is one of the most popular floriculture in the world ornamental and flower market. This species is belonged to *Curcuma* genus and is native to Indo

China including Thailand, Laos and Myanmar (Gagnepain, 1908). They are common on the plateau of North Eastern Thailand and their distributions are extended to the coast of low land of Thailand and West Cambodia (Paisooksantivatana *et al.*, 2001).

2.2 Morphological characterization of *C. alismatifolia*

2.2.1 Roots system

C. alismatifolia has a succulent adventitious roots system, which can be separated in two types. The first type is a group of 'fibrous roots' that develops from storage roots and from the basal of new shoot as soon as rhizome sprouting (Hagiladi *et al.*, 1997). The second one is the 'contractile roots' which also initiates from the basal of new shoot and develops into storage roots (spherical shape) when aerial part dies down (the end of growing season) (Chidburee, 2008; Hagiladi *et al.*, 1997).

2.2.2 Underground organs

The underground part of the plant bears two types of storage organs, i.e. 'rhizomes' and 'storage roots'. Both organs are fleshy and have different function for growth and development.

Rhizome

Rhizome is an organ which modified from the vertically growing stem with short internodes (stubbed rhizome). It comprises of buds, nodes and internodes.

An active lateral bud of vivacious rhizome will sprout and develop to a new shoot which, in turn, will generate leaves and inflorescence for the coming season.

After flowering, the formation of new rhizomes occur from the base of swelled pseudostem (Khuankaew, 2010). Rhizomes with attached storage roots have better keeping quality under the post-harvest condition than those without them (Phubuopuen, 1992; Wannakrairoj, 1996). However, rhizomes without storage roots can also produced flower lately (Hagiladi *et al.*, 1997b). When flower ceases, the aerial organs will dry and the rhizomes will become dormant.

Storage roots

Storage roots are modified from enlarging contractile roots that connecting to rhizomes. The terminal end of contractile roots are swollen and become spherical in shape. The storage roots play an important role as a storage organ which contain high water content and food reserve, particularly carbohydrates (Khuankaew *et al.*, 2009; Ruamrungsri *et al.*, 2001). The number of storage roots present are thought to have an impact on plant sizes and flowering times (Hagiladi *et al.*, 1997b).

2.2.3 Aboveground organs

Leaves

The leaves of *C. alismatifolia* consist of '**leaves sheath**' that are tightly wrapped and compact, so call the '**pseudostem**', and '**leaves blade**' which are usually elliptic (oval and flat with narrowed to each end round shape) with penni-parallel (parallel veins run along the leaves length) and strongly ascending veins (Gerald, 1997). The size of leaf blade is approximately 4-5 cm wide, and 30-35 cm long, and is mostly deep green color with reddish median nerve (Phubuopuen, 1992). Leaves are oppositely arranged in a flat, two dimensional planes (Phongpreecha, 1997).

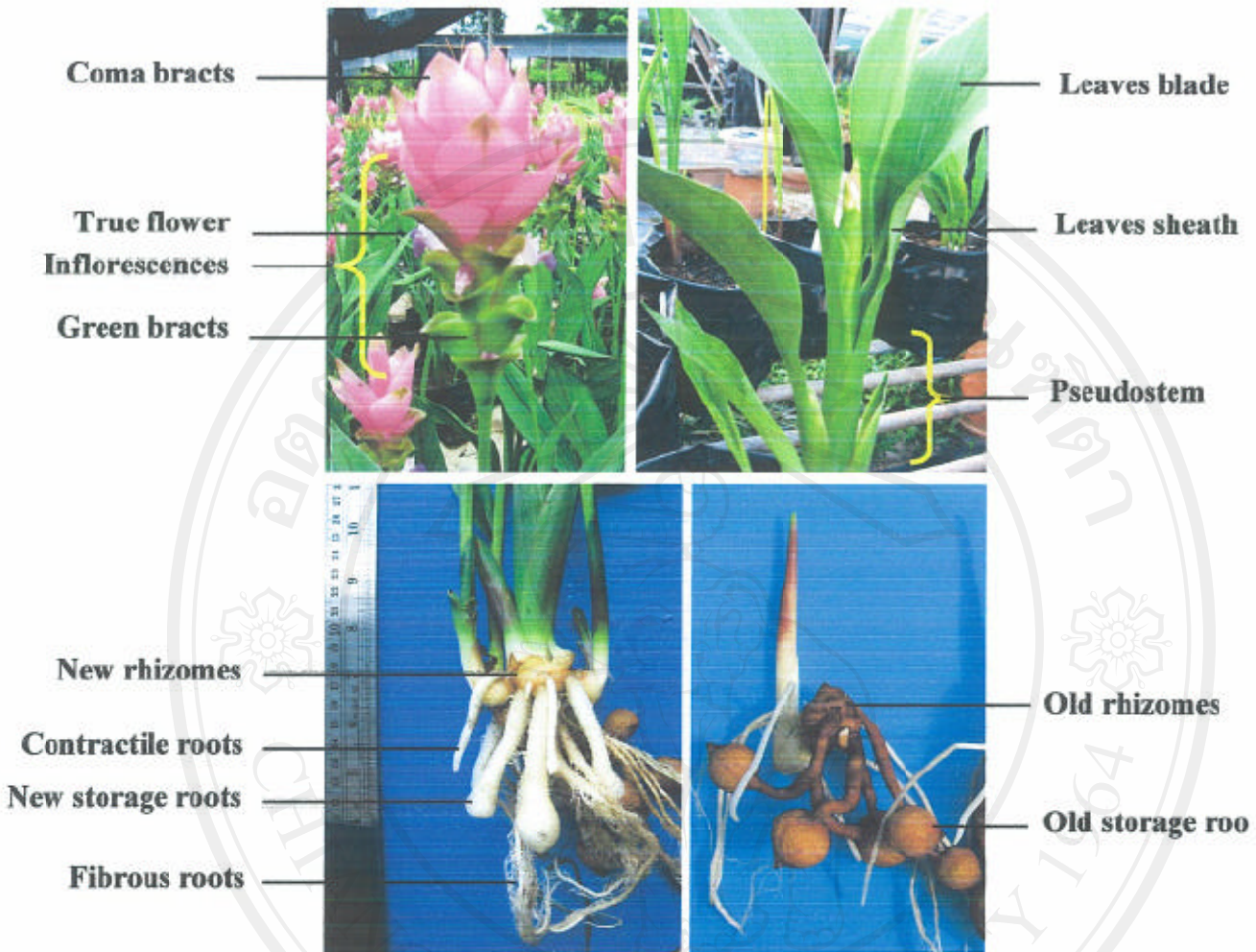


Figure 2.1 Morphological characterization of *C. alismatifolia* Gagnep.

Pseudostem

Pseudostem comprises of basal foliage leaves (leaves sheath) tightly enfold together around inflorescent stalk.

Inflorescence

Inflorescence is showy compact spike, bears with prominent spiral green bracts, which are laterally fused, forming into pouches at lower parts. The green

pouch also subtends 2-10 'true flower' (florete) which opposite the pinkish pale bracts (coma bracts) in upper part. The comprising of long lasting bract shape and their often bright color made them look like lotus flower (Wannakrairoj, 1996).

Flower

The true flowers that hide in the axils of green pouches are bisexual, strongly zygomorphic, and often are associated with conspicuous floral bracts in a spike. The perianth is in 2 whorls, the first one is spathaceous tubular calyx and the second one is a petaloid tubular corolla with 3 lobes. The androecium typically consists of 1 fertile stamen, a large opposing petaloid labellum representing 2 connate staminodia, and two smaller flanking petaloid staminodia. The gynoecium consists of a single compound pistil of 3 carpels, a single style nestled in a channel of the filament and anther of the fertile stamen and an inferior ovary with typically 3 locules, each containing numerous axile ovules. Rarely, the ovary is unilocular with parietal placentation (Gerald, 1997)

2.3 Growth cycle

Generally, most *C. alismatifolia* are planted in rainy season. The stored rhizomes, with 4-5 storage roots, are planted in April or May. Flowering starts at 10-12 weeks after planting (WAP) and continues until the end of September. By October, aerial parts are wilted and underground parts entered into dormancy in November to December during cool dry season (winter) (Wichailak, 2005)

2.3.1 Vegetative growth

The growth of *C. alismatifolia*, as other bulbous plant, usually is not continuous. It occurs as intermittent, short lasting shoot sproutes from active lateral buds of resting modified stem (rhizomes) before returning to a quiescent state of new rhizomes in next season. Vegetative shoot is sprouting, either from lateral buds, at 1-2 weeks after planting (WAP). Shoot grows and develops to 1st foliage leaf takes a time for 2-3 WAP. Generally, plant produces 3-5 fully expanded leaves per shoot before flowering at 8-10 WAP (Khuankaew *et al.*, 2009).

The plant height (foliage leave length) of the 1st leaves rapidly increases from 3-5 cm at 2 WAP to 26-30 cm at 4 WAP and reaches a maximum at 6 WAP. However, the 1st mature leaves length is usually shorter than the 2nd and 3rd leaves. Development of the vegetative shoot from initiation of growth to full elongation takes 2-6 WAP, depending on the seasonal cropping and climatic conditions (Khuankaew *et al.*, 2009).

2.3.2 Reproductive growth

The terminal inflorescences are initiated from lateral buds on modified stem (rhizomes) that developed vegetative growth and followed by the flowering. The first sign of generative inflorescences initiation in lateral bud is the swelling of flattened axillary meristem, which is developed into a dome shape when plant reaches 6-10 cm in height (Changjeraja, 2009). When they are 11-15 cm in height, the plants start to differentiate the inflorescence by enlarging of bud in bract axil and depressing flower

of primordium center for first true flower (flore) initiation. *C. alismatifolia* starts to form the floral organ when they reaches 16-20 cm height (Changjeraja, 2009). It takes 2-3 WAP from inflorescence initiation to inflorescence differentiation.

Flower stalk with a small inflorescence is bolted and covered with several foliage leaves at 9 WAP. The 1st floret is opened at 10 WAP and fully bloomed at 11-12 WAP. The base of flower stalk is connected with a new rhizome which is developed from the basal leaves sheath. Then, 1 to 2 new shoots are sprouted from old rhizome at this stage and contractile roots are emerged from the basal part of new rhizome. At 20 WAP, the first inflorescence ceases with the 1-3 new shoots per plant are produced and the terminal of contractile roots become swollen, and develop into new storage roots which cause by cell division and cell enlargement in the cortex and stele (Chidburee, 2008). Leaves and inflorescences senesce at 24 WAP and new rhizomes with new storage roots become dormant. At this time, new rhizomes, with attached storage roots, are harvested. One old rhizome can produce up to 4-5 new rhizomes with new storage roots. The old rhizome becomes fading and dry with deep brown in color.

2.4 Regular season and off-season cropping

2.4.1 Regular season cropping

Generally, regular season cropping of *Curcuma* in Thailand can be categorized in three periods, namely:

Early-season Cropping:

Rhizome must be treated for pre-germination before planting by soaking them in water to stimulate shoot emergence. Cropping starts from February to March. Early flowering occurs in May and harvesting of rhizomes can start in December (Wichailak, 2005).

Mid-season Cropping:

Planting is made during April and May depending on regular rainfall. *C. alismatifolia* will flower during July and August (about 8 to 24 WAP) and will dormant in November to December in the cool dry season. Rhizomes are harvested in December and sold for export in January (Wichailak, 2005).

Late-season Cropping:

Rhizomes are planted from late June to July. The advantage of this late season planting is that the soil has adequate time to dry in the sun, and to control soil borne pathogens, resulting in minimizing the risk of infections but the rhizome has only 5-6 months to accumulate reserve food. Management of adequate water and fertilizer is crucial. The harvested rhizome has short swollen root that reserve food, which is the most suitable for export. The rhizome must be carefully kept in order to avoid drying out before planting (Wichailak, 2005).

2.4.2 Off-season cropping

The proposes of this off-season cropping are: (i) to avoid surpluses of the regular season cut flower or rhizome production, to avoid danger of epidemics, and to increase profit for farmer, (ii) to satisfy customers at their demands; and (iii) to guarantee employment throughout the year. In *C. alismatifolia*; regular season flowers are produced during the rainy season (from June to August), requiring long day condition. The decreasing of flower quality is occurred after September when short day condition commenced. All of the above ground parts wilt and die down, and rhizomes enter dormancy period until next rainy season. Supplemental lighting by giving 2 h of incandescent light could improve flower quality and rhizome yields in off-season cropping of *C. alismatifolia* (Ruamrungsri *et al.*, 2007).

2.5 Effects of endogenous hormones on growth and development

2.5.1 Auxins

The natural auxin is indole-3-acetic acid (IAA). IAA is one of the principal hormones that control plant growth and development. In combination with the other hormones, it is involved in embryo genesis, all type of organogenesis, root meristem maintenance, vascular tissue differentiation, hypocotyls and root elongation, apical hook formation, apical dominance, fruit ripening and growth responses to environmental stimuli, i.e. phototropism and gravitropism (Vieten *et al.*, 2007). The highest IAA content is found in regions of active cell division, such as apical meristem, the cambium, the developing fruit, in embryo and endosperm. All younger plant parts seem to be able to synthesize IAA (Ljung *et al.*, 2001). In higher plant,

IAA is synthesized by multiple pathways. In some case, tryptophan serves as the precursor, whereas in others, the immediate precursors of tryptophan and indole-3-glycerol phosphate seem to give rise to IAA by parallel pathways (Gruber and Bangerth, 1990). Regulation of growth in plants may depend in part on the amount of free auxin present in plant cells, tissues and organs. Levels of free auxin can be modulated by several factors, including the synthesis and breakdown of conjugated IAA, IAA metabolism, compartmentation, and polar auxin transport (Taiz and Zeiger, 1998).

IAA is transported basipetally and acted as a hormonal signal, when it is transported from an organ or tissue to another, different from the movement of sugars, ions and other certain molecules. IAA levels in the apoplast solution is higher than in the symplast, and that young leaves exports the large amount of diffusible IAA than older young leaves (Kojima *et al.*, 2002). However, the translocation of IAA is not preferably through the phloem sieve tubes or through xylem, but instead by a highly specific cell to cell transport in specialized phloem parenchyma cells which are communicated with vascular bundles (Ross, 1992). Diffusible hormones may be more relevant to the physiological phenomena than extractable hormones (Sjut and Bangerth, 1984).

2.5.2 Abscisic acid (ABA)

ABA, a plant hormone and growth regulator, is ubiquitous in higher plants and has many physiological effects on growth and differentiation of plants (Zeevaart and Creelman, 1988). ABA also presents widely among different organs, i.e. growing regions like young leaves. In general, leaves have higher ABA content than roots, and developing seed and ripening fruit are usually rich sources of ABA (Zeevaart *et al.*, 1999). Previous studies have been reported that ABA may be involved in determining longer term patterns of biomass partitioning (Creelman *et al.*, 1990; Nagel *et al.*, 1994; Saab *et al.*, 1990). However, ABA is also involved in numerous physiological processes, such as stomatal regulation by its strongly promotes stomatal closure and inhibits stomatal opening (Liu *et al.*, 2005; Serraj *et al.*, 1999), the stability of photosynthetic apparatus (Gong *et al.*, 1998) and controls various organ's dormancy and dormancy release (Okubo and Uemoto, 1981). ABA has been implicated in growth cessation and senescence in combination with ethylene (Panavas *et al.*, 1998), through its ability to induce senescence associated with lipid peroxidation and ion leakage, as well as, the appearance of protienase and ENase activity (Panavas *et al.*, 1998). ABA is also functioned as hormonal response to environmental stress or biotic factors, such as drought, cold, heat and salinity (Zeevaart *et al.*, 1999). Nevertheless, a common functional mode of ABA signaling (root signaling) in drought stress, it has been proposed that ABA is produced in root tips and transported into xylem through the transpiration stream reaching the leaf where it reduces stomatal conductance (Davies *et al.*, 2002; Liu *et al.*, 2005). This leads to closure of stomata to prevent water loss by transpiration and to synthesis of specific proteins and compatible

osmolytes that protect the cellular membranes and macromolecules from damaging effects of dehydration (Srivastava, 2002). Moreover, ABA responses are also associated in storage organ's forming in combination with low temperature, short day length, low rate of N fertilizer and balancing other plant hormones (low GA and high CK) (Krauss, 1985) and it can also regulates the thickening and dry matter accumulation in some tuberous plant (Nakatani and Komeichi, 1991).

2.5.3 Cytokinins (CKs)

Cytokinins have been known as key substances in hormonal regulation of plant development. The natural forms are a series of adenosine molecules which modified by the addition of 5-carbon side chains at the 6th carbon position. They have a broad spectrum of biological activities involve in cell division, shoot-root formation in callus, apical dominance, xylem formation, leaf senescence, root growth and cotyledon expansion (Hooykass *et al.*, 1999). CKs is also involved in the formation of storage organs by controlling the rate of meristematic cell differentiation (Kyozyuka, 2007; Dello Ioio *et al.*, 2007), sink creation by regulating the expression of gene implicated in assimilate partitioning and participation in source-sink relation (Roitsch and Ehneß, 2000). It is widely accepted that root tips are the major site of CKs biosynthesis and it is translocated through the xylem to control shoot development. Nevertheless, it has been indicated that the shoot transmits signals, probably IAA, to roots and thereby controls processes involved in the regulation of CKs biosynthesis in root (Bangerth, 1994; Beveridge *et al.*, 1997). On the other hands, there is some

evidences to show that meristematic tissues other than root meristems are also capable of CKs biosynthesis (Komor *et al.*, 1993) and leaves can also be rich in CKs (Hooykass *et al.*, 1999). Translocational form of CKs is *trans*-Zeatin riboside (*t*-ZR) (Hewett and Wareing, 1973; Gordon *et al.*, 1974; Letham, 1974) which is the dominant CKs in xylem sap of most plants analyzed. This CKs form is transported from root to leaves via the transpiration stream (Shashidhar *et al.*, 1996; Hare *et al.*, 1997). The dominant CKs transported in phloem sap of *Richinus communis* seedlings has been shown to be isopentanyl adenine and zeatin (Baker, 2000; Komor *et al.*, 1993), while isopentanyl adenosine and zeatin riboside dominated in xylem exudates.

2.5.4 Gibberellins (GAs)

Gibberellins (GAs) affect almost every aspect of plant growth and development. Their most notable property is the enhancement of stem growth through cell elongation. Other roles include promotion of α -amylase synthesis in cereal as seed germination and florigenic activity (Katekar, 1999). GA biosynthesis is particularly active in immature seeds, especially in the endosperm, where it is transported α -amylase from aleurone cell which is induced by GA. The synthesis starts from mevalonic acid through the hydrocarbon *ent*-kaurene (Kendrew and Lawrence, 1994) which still retains basic structure of its extensive chemical family. GA can be defined according to the structure rather than its biological activity (Srivastava, 2002). In reproductive development, GA can affect the transition from juvenile to the mature stage, as well as, floral initiation, sex determination and fruit

set. However, GA can substitute for the long-day or cold requirement for many plants, especially rosette species that require either long days or low temperatures to induce flowering (bolting phenomena). GAs may also play the role in the regulation of tuber and bulb formation as the balancing of other hormones, in combination with short day length and low temperature condition (Krauss, 1985). GAs accumulating actively under long day unfavorable for tuberization, are known as most strong inhibitors of tuber formation. But, according to the information until now, might indicating the complex of GA action on tuber development by their different forms exert differently and some time opposite action (Aksenova *et al.*, 2009). However, it has been demonstrated that the distribution of different GA forms, especially GA₂₀ and GA₁, between potato organs plays an important role in tuber initiation (Bou *et al.*, 2003). GAs are most often associated with the promotion of stem growth by cell elongation and the application of GA to intact plants can induce large increase in plant height. In *C. alismatifolia*, the GA₃ application could promote shoot height, flower stalk and also delay the flowering date (Khuankaew *et al.*, 2008a; Khuankaew *et al.*, 2008b).

2.6 Environmental factors affect endogenous hormones levels

Plant growth and development is tightly co-ordinate with surrounding environment. This adaptive plasticity is achieved through the complex integration of physiological parameters. Light, temperature and gravity have the most obvious and dramatic impact. Other environment factors, such as soil moisture and nutrition, may

also influence development in some cases. More recently, it has become evident that varying of air and water pollutants may significantly modify developmental patterns.

Because environmental signals originate outside the plant, plants must have some means of perceiving the signal and converting or transducing the information in some permanent metabolic or biochemical changes. It is also becoming increasingly evident that environmental stimuli act, at least in part, through modifying gene expression or hormonal activities (Hopskins, 1999). Complex physico-chemical changes, including the balance between endogenous phytohormones, lowering the levels of some and raising others, are the results of different environmental variables. The environmental variables have great influence on the endogenous levels of several types of phytohormones. For instances, constituent of the phytohormones changes occur under low and high temperature, light, drought and salinity (Die and Campbell, 1981; Walker and Dumbroff, 1981; Chen *et al.*, 1983; Srivastava, 2002).

2.6.1 Temperature

Temperature is an important seasonal cue which enables plant to predict and consequently prevent the adverse effects of environmental change. Plant responses to temperature are twofold. Temperature signals can act as a stimulus to control the timing of developmental transitions and to enhance tolerance to future temperature extremes (Franklin, 2009). Recent studies have also demonstrated temperature signals in regulation of germination, plant architecture, flowering and enhancements of freezing tolerance by evoking changes in hormone levels (Franklin, 2009).

Temperature and flowering

Temperature is considered as an importance environmental factor responsible for both flowering induction (Vaz *et al.*, 2004) and changes in endogenous phytohormone in some tropical orchid plants (Goh, 1979). A decrease in cytokinin has been observed in *Phaleanopsis hybrid* which grown under high temperature, a condition that promoting vegetative growth (Chou *et al.*, 2000).

Campos and Kerbauy (2004) reported the increasing of IAA and cytokinin, especially the zeatin-derived forms in bud of *Dendrobium* 'Second love' under the high temperature condition (25°C), while the amount of ABA decreased progressively throughout the treatment and suggested that hormones were involved in the signal transduction pathway of thermoperiodic flowering control. However, Fouch *et al.* (1997) observed the fluctuating levels of IAA in the leaves of *P. ambillis* after the inductive thermoperiodic treatment, but it was not certain whether it was related to flowering.

In some angiosperm woody plants, cool temperature is the factor which can also regulate the reproductive development (flower induction or initiation) and may be related endogenous hormonal changes. Naphrom (2004) reported the higher CKs, lower GA in all plant tissues and lower IAA in vascular tissue of mango tree under the cool temperature treatment when flower induction occurred.

Temperature and stress

Extreme temperatures appear to be intrinsic, often differences between species, which involve in tissue adaptive response for stress conditions, such as freezing and drought. ABA also plays a role in response of plant to adverse environment. Several types of evidence have suggested that ABA controlled processes are central to cold acclimation and development of freezing tolerance in woody plants by altering their levels paralleled with this phenomena (Li *et al.*, 2003; Li *et al.*, 2004). Early reports have revealed that low temperatures can increase endogenous ABA levels (Baldwin *et al.*, 1998; Li *et al.*, 2004; Li *et al.*, 2002).

Temperature and dormancy

Dormancy is a survival strategy for the organisms that displays it and enables the plant to resist unfavorable environment. For species of the temperate zone, low winter temperatures are a major environmental factor and one of the important accompaniments to winter dormancy is a marked increase in the resistance to below freezing condition (Thomas and Vince-Prue, 1997). Low temperatures also induce dormancy in bud and seed that is characterized by low respiratory rate and inhibition of growing even if temperature, oxygen and water supply are adequate. However, endogenous ABA levels are associated with dormancy development and release. Many dormant buds and seeds have chilling requirements for dormancy breaking to renew growth. Most ornamental bulbs generally develop dormancy from late autumn to winter and dormancy can be released by low temperature (Langens-Gerrits *et al.*, 2003). In lily bulbs, a cold treatment triggers the metabolic activity within the bulblet

of lily (Shin *et al.*, 2002), which leads to sprouting and plant development. The correlation between decreasing ABA and dormancy released under the cold storage is pronounced in many bulbous plants (Naor *et al.*, 2008; Xu *et al.*, 2006; Yamazaki *et al.*, 1999).

Temperature and vernalization

Vernalization is referring specially to the promotion of flowering by a period of low temperature (Salisbury and Ross, 1992). Temperatures of approximately 0 to 5°C, apply to the active growing apex of the plant for several weeks, is required for effective vernalization. Application of GAs normally substitute for the cold requirement in vernalization by promoting flowering (include bolting) in some long day plants and stem elongation in some short day plants. Both responses are the separate processes, but closely linkage genes. Vernalization can be reversed if followed immediately by a high temperature treatment which also calls ‘**de-vernalization**’ (Hopkins, 1999).

2.6.2 Light

Light is used by plants not only as a source of energy for photosynthesis, but also as a signal for growth and morphogenesis, including seed germination, seedling development, morphological and physiological processes of the vegetative stage, the control of circadian rhythms and flowering (Kim *et al.*, 2003; Nemhauser and Chory, 2002). Light is also a very versatile signal, which varies not only in quality (wave length) but also in quantity (fluency or photons m⁻²), duration (photoperiod or day

length) and direction (unidirectional or diffuses). These multiple facets of the light signal are overlaid by periodic phenomena which emanate from the earth's radiation around its axis (diurnal periodicity) and around the sun (annual or seasonal periodicity). Plants perceive these varying signals by a variety of photoreceptors and integrate that information to modify their growth and development and to time their reproductive strategies (Srivastava, 2002). However, many changes of the light-induced during growth cycle are also known to be regulated by phytohormones (García-Martínez and Gil, 2001) such as the direct regulation of the biosynthesis of active GAs (Oh *et al.*, 2006; Seo *et al.*, 2006) and ethylene (Steed *et al.*, 2004) and controlling the degradation of ABA through the transduction pathway of phytochrome signal, both at the tissue and organism levels (Kraepiel *et al.*, 1994). Thus, the integration of light and hormone signaling pathways is also thought to be required for normal plant development (Clouse, 2001). Photoperiod is the amount of light and darkness in a daily cycle of 24 h. The different day length and night length becomes more extreme in experienced at certain times of the year. The annual rotation of the earth around the sun causes the photoperiods at particular latitude to change throughout the year, with day length becoming longer in summer and shorter in winter (Jackson, 2009). The annual cycle of variation in photoperiod is consistent from year to year and it is thus a reliable indicator of the time of year, much more reliable than the temperature which also shows seasonal variations, but is far less predictable.

Light and flowering

Light, in terms of 'duration' or 'photoperiods', has direct influence in flowering process. The temperatures and photoperiods also affect flower initiation and development in many species. The requirement for exposure to a particular photoperiod in order to affect flowering can be profoundly modified by temperature and *vice versa* in orchid plants (Vaz *et al.*, 2004).

Photoperiod has been shown to influence the content of GAs in shoot tip when floral evocation occurs. In *Silene*, the levels of several 13-hydroxy GAs are higher in the tips of long day induced plant than in short day, especially in the meristematic zone approximately 0.5-1.5 mm below the apical meristem (Talon *et al.*, 1991). GAs may act either in the leaf, or at the apex. In general, there appears to be increased amount and/or turnover of bioactive GAs in long day condition, and this may be associated with the regulation of flowering by day length. The increased GA content under long day condition may be necessary for synthesis of floral stimulus in leaves of a long day plant (*Hyoscyamus*). In rosette long day plants, the increased GA content under long day condition essential for stem elongation normally accompanies flowering in these plant (Thomas and Vince-Prue, 1997).

Photoperiods has been shown to control the levels of CKs in *Chinopodium* species, with the amount in leaves, stems and roots decreasing during the dark periods and increasing again during the light periods (Machackova *et al.*, 1993). Analysis of phloem exudates in which the amount of exported CK from the leaves of *Perilla* is increased by exposure to inductive short day, also suggests that CK may be part of a

floral stimulus, in some case at least, may be a limiting factors for flowering (Thomas and Vince-Prue, 1997).

The endogenous auxin content (mainly based on bioassay) has been found to vary in different photoperiods, but no consistent trends have been observed (Thomas and Vince-Prue, 1997) and the role of endogenous auxins in photoperiodic regulation of flowering remains obscure. However, it has been suggested that auxin may be an endogenous inhibitor of flowering providing a general background of inhibition, which the stimulating effects of photoperiods, can overcome (Jacobs, 1985).

In the case of seasonal variations, there have been some reports to show that long day condition (artificial extended photoperiods) causes the higher ABA levels in the xylem sap, mature leaves and apices of willow (*Salix viminalis* L.) than those grown in natural day length (Alvim, 1978).

Light and plant acclimation

CKs may play an important role in photoperiod improvement of plant growth under environmental stress (Pons *et al.*, 2001). An extended light period increases CK levels in long day plants, such as *Sinapis alba* L. (Lejeune *et al.*, 1988), , tomato (*Solanum tuberosum*) (Machackova *et al.*, 1998) and creeping bentgrass (*Agrostis stolonifera* L.) (Wang *et al.*, 2004). A study with long day plant, *S. alba*, and short day plant, *Xanthium strumarium* L., suggests that the cytokinin synthesis is photoperiodical controlled and may serve as root to shoot signal in affecting shoot growth and development (Kinet *et al.*, 1993).

Light and etiolate phenomena

Light has the influence on plant growth and development, perhaps most obvious during the transition from a dark-grown (etiolated) to light-grown (de-etiolated) morphology. Etiolated dicotyledonous seedling exhibits a phenotype upon the light, such as the reduction of shoot elongation rate, opening of the apical hook, expansion of true leaves and development of mature chloroplast (Chory *et al.*, 1996; Clouse, 2001). A clear evidence exists for a light-mediated reduction in GA levels and their response in pea as reduced shoot elongation (O'Neill *et al.*, 2000; Reid *et al.*, 2002). Red light appears to control GA₁ levels by down-regulating the expression of Mendel's *LE* (*PsGA3ox1*) gene that controls the conversion of GA₂₀ to GA₁, and that by up-regulating *PsGA2ox2* which codes for GA₂-oxidase that converts GA₁ to inactive GA₈ (Reid *et al.*, 2002). These changes in gene expression occur when exposure to the red light and precede changes in endogenous GA₁ levels.

However, physiological significance of light-induced changes in IAA and ABA levels found in some species (Chory and Li, 1997; Tian *et al.*, 2002) is not clear. But, the recently molecular studies of light and auxin transduction in *Arabidopsis* have produced strong evidences that light may modulate auxin signaling pathway (Tian and Reed, 2001). Some reports have shown that light may normally regulate the expression of the *AUX/IAA* gene (*AXP2*, *AXP3* and *SHY2*) with the occurrence of photomorphogenic characteristics in dark-drown seedlings (Colon-Carmona *et al.*, 2000; Tian and Reed, 2001). However, light induced the decrease in IAA levels may contribute to the inhibition of plant cell elongation after being exposed to the light (Kraepiel and Miginiac, 1997). While, the ABA levels in etiolated wild type plant

gradually decrease and reach a minimum 48 h after exposure to light (Kraepiel and Miginiac, 1997). Indeed, the timing of the decrease in ABA levels in de-etiolating can be a consequence of the changing morphology after exposure to the light (Symons and Reid, 2003).

Light and storage organs formation

The formation of storage organs (tubers and bulbs) depends substantially on photoperiodic condition and hormonal balance (Ewing, 1995). The stimulus is synthesized in leaves and transited the developmental information to the target site, where the morphogenetic events take place (Thomas and Vince-Prue, 1997). The photoreceptor for the perception of day length is phytochrome, especially, phytochrome B, and the actual mechanisms of short day sensing for tuberization and long day sensing for bulbing show marked similarities to the day length sensing mechanisms for the photoperiodic control of flowering (Thomas and Vince-Prue, 1997). Tuberization in potatoes is a short day dependent process which affects substantially to plant hormone status (Ewing, 1995). Since, phytohormones, GAs and CKs, primarily affect considerably on tuber formation by imposing their effect on the hormonal balance. GAs, actively accumulating under long day unfavorable for tuberization, are known as strong inhibitors of tuber formation (Vreugdenhil and Sergeeva, 1999). However, it has been found that different GA forms exert different and sometimes opposite action in regulating tuber formation (Bou *et al.*, 2003; Xu *et al.*, 1998). It has been demonstrated that a distribution of different GA forms, especially GA₂₀ and GA₁, between potatoes organs plays an important role in tuber initiation, and CKs are believed to be stimulators of tuber initiation and growth

(Hannapel, 2007) since, tuber initiation is related to cell division and enhancement of sink organ activity (Roitsch and Ehneß, 2000). However, some reports have shown that CK role in tuberization depends on its predominant localization in the above or underground potatoes organs, and this may be involved in the competitive relation of GA and CK between growing tuber and shoots (Aksenova *et al.*, 2009).

2.6.3 Mineral plant nutrition

Plants count on a wide variety of metabolic, physiological, and developmental responses to adapt their growth to variations in mineral nutrient availability. Mineral nutrient conditions influence balancing of phytohormone via the different changes in their yields response during certain periods of plant development (Marschner, 1995) and their biosyntheses, i.e. the interrelation between hormonal stimuli and nutritional homeostasis (Rubio *et al.*, 2009).

There are several reports suggesting that the accumulation of CK is closely correlated with the nitrogen (N) status of the plants; such as *Urtica dioica* (Wagner and Beck, 1993), barley (Samuelson and Larsson, 1993) and maize (Takei *et al.*, 2001). These studies suggest that CK metabolism and translocation could be modulated by N nutritional status. A remarkable finding is that, the increase in CK concentration occurs following the change of N status from deficient to sufficient (Samuelson and Larsson, 1993; Takei *et al.*, 2001). In maize roots, following the addition of nitrate to nitrogen-depleted maize plants, iso-pentenyladenosine-5'-monophosphate (iPMP) begins to accumulate in roots within 1 h, preceding

accumulation of *trans*-zeatin riboside-5'-monophosphate (ZMP), *trans*-zeatin riboside (ZR) and *trans*-zeatin (Z) (Takei *et al.*, 2001).

Ammonium (NH_4^+) and nitrate (NO_3^-) are the two form of N available for plant growth. Most plant species supplied with NH_4^+ and NO_3^- forms can be more productive than those supplied with NH_4^+ or NO_3^- alone (Wang and Below., 1996). It has been found that the levels of CK in both the shoot and the root of mixed-N-grown plants are higher than those of two N form alone grown plants. ABA levels are similar among plant grown under different N forms. While, the mixed-N-grown plants have been shown the higher GA in shoot, but the lower IAA in shoot (Chen *et al.*, 1998).

It is well known that nitrate is the major form of available inorganic N in most agricultural soil. Some reports have shown that nitrate itself uses cytokinin as a messenger. Plant uses nitrogen signaling routes for communicating internal and external N status and it specifically regulates a wide variety of metabolic processes including nitrogen and carbon metabolism and cytokinin biosynthesis (Sakakibara *et al.*, 2006).

Phosphorus (P) is one of the essential macro nutrients which play basic functions in plant metabolism and physiology as constituents of metabolites and macromolecules. Its assimilated form inorganic phosphate (Pi), is part of nucleic acids, phospholipids, and proteins, and it plays an essential role in energy transfer reactions and signaling cascades, and acts as an electrolyte (Marschner, 1986; Marschner, 1995)

Plants can acclimate to P deficiency also by increase in the root-shoot mass ratio or root surface by the formation of more dense root hair which is the result of

shoot growth reduction and stimulation of root growth. P deficiency symptoms has been related in auxin, ethylene and cytokinin responses for morphological adaptation process of plant (Wittenmayer and Merbach, 2005). Low Pi availability increases the number of root hairs, tip elongation and development process, where it is believed to be controlled by auxin and ethylene (Schiefelbein, 2000). However, ethylene has been indicated as regulator of root architecture responses to Pi starvation, frequently resemble that causes by treatment with ABA, and transportation of ABA seems to be stimulated in Pi-deficient plant (Wittenmayer and Merbach, 2005).

Boron (B) is suggested to have primary effects relate to the cell wall and plasma membrane extensibility (Marschner, 1986; Marschner, 1995). One of rapid response to B deficiency is the inhibition or cessation of elongation growth of both primary and lateral roots. Some reports have shown the elongation root growth is inhibited in *Cucurbita pepo* L. with the dramatic increase in the activity of IAA oxidase (Bohnsack and Albert, 1977). However, auxin levels of B deficient plant are often much higher than normal (Coke and Whittington, 1968). B deficient has been found to release apical bud dominance and reduce IAA and Z/ZR type of cytokinin concentrations in the pea shoot apex (Li *et al.*, 2001). While, there has been found the reduction in auxin and CKs (Z/ZR, iso-pentanyladenine: i-Ade/i-Ado) in B deficient plant and the decreasing of IAA export from shoot apex when supplies with B. Application of B to shoot apex restores the endogenous Z/ZR and IAA levels and increases the IAA export from shoot apex of pea (Wang *et al.*, 2006). This suggests that the complex interaction between plant hormones, with a B deficiency induced inhibition of IAA export from the shoot apex as one of the earliest measurable event.

Calcium (Ca) is a relatively large divalent cation (Ca^{2+}) which readily enters the apoplast and is bound in an exchangeable form to cell wall at the exterior surface of the plasma membrane. In cell extensibility, auxin may play a role involving in Ca^{2+} transport within plant tissue and the inhibition of Ca^{2+} transport or a decline in the levels of auxin (Marschner, 1986). Moreover, auxin-induced enhancement of proton excretion, a prerequisite for the wall-loosening process and thus for cell extension, requires exogenous Ca^{2+} (Cohen and Nadler, 1976). Nevertheless, Ca^{2+} is ubiquitous secondary messengers in living plant cell. In stomatal guard cells, one of the best-characterized plant cell models, increasing cytosolic free Ca^{2+} concentration is known to inactivate inward rectifying K^+ channels in plasma membrane for solute efflux which drives stomatal closure (Blatt and Grabov, 1997). Changes in cytosolic free Ca^{2+} concentration have been associated with the stimulus ABA. Ca^{2+} selective channel which can account for Ca^{2+} influx and increasing of cytosolic free Ca^{2+} can be triggered by voltage and ABA (Hamilton *et al.*, 2000). In the case of α -amylase, the effect of Ca^{2+} depends on the present of phytohormone GA (Jones and Carbonell 1984) and is more likely a reflection of a Ca^{2+} / calmodulin induced enhancements of synthesis and secretion of this enzyme by the cells (Mitsui *et al.*, 1984).

There has been a report to show that Mn^{2+} deficient plant exhibits the highest levels of IAA oxidase activity with the leaf abscission and reduced growth in young tissue of cotton (*Gossypium hirsutum* L. cv. Deltapine) seedling in culture solution and relates to destruction of auxin (Morgan *et al.*, 1976).

2.6.4 Water

Water is the one of the most important ingredient of life. Biological function is completely dependent upon water, and plant requires water for its utilization in various metabolic processes going on in living cell (Salisbury and Ross, 1992). More than 80-90% of most plant cells and tissue is constituted by water. It forms a continuous liquid phase through the plant from the root hairs to the leaf epidermis and continuity generally extends into the soil or substrate (Sinha *et al.*, 2005). The relationship between soil-plant-atmosphere in water movement has been emphasized as the flow of 'water potential' with its various components (osmotic, matrix and pressure) (Kramer, 1969). This potential is widely used to describe the free energy or chemical potential of water in soil, plant and air system.

Water stress in plant is the phenomena terms to describe the ability of plant to adaptation in the sense of strenuous efforts undertaken to mobilize this defense system for adverse condition of water, such as deficit or excessive water levels (Chernyad'ev, 2005). When plants are subjected to abiotic stress, some of endogenous phytohormones have been found to be the key elements involved in signal transduction and in regulation of gene expression in plant responses to drought. Under conditions of water stress, a rapid accumulation of ABA results in stomatal closure, synthesis of specific proteins and mRNAs, regulation of physiological process and improved ability to adapt to water stress (Chinnusamy *et al.*, 2004; Verslues and Zhu, 2005; Xiong *et al.*, 2002). In micro propagated apple tree (*Malus domestica* Borkh cv. Nagano Fuji), water stress condition induces the leaves to produce large amount of endogenous ABA, sucrose phosphate synthase activity (SPS) and aldose-6-phosphate

reductase (A6PR). This indicates the role of ABA in the regulation of carbon distribution in photosynthesis under water stress (Li and Li, 2007).

The synthesis of cytokinin in root tissue declines and its upward transportation decreases with the increase in water stress (Itai *et al.*, 1968). This can be benefiting to the plants under drought conditions by decreasing the conductance of the stomata and the velocity of evaporation, thus maintaining water balance (Jackson 1990). Some reports have shown that moderate water stress, by decreasing (25%) irrigation levels, lead to the increase in DHZR and *t*-ZR concentration in xylem sap of lychee (*Litchi chinensis* Sonn.) with induced fruit bud differentiation (Stern *et al.*, 2003).

Growth reduction under drought stress is mainly caused by a decrease in a concentration of IAA (Saugy and River, 1988). Nevertheless, there are reports that there are no significant changes in IAA under drought stress (Li *et al.*, 2000) and that changes in IAA caused by drought have no significant regulatory function in the process of adaptation of a plant to drought.

Flooding or water logged, the terms to describe soil with excessive water levels, is a major environmental stress that can severely decrease crop growth and productivity. It can be the causes for mineral deficiency, an increase in toxic compounds in the soil, an increase in organisms, hormone imbalance (Liao and Lin, 2001). Changes the relationship between root and shoot hormones occur when plant faces flooding, e.g. decreasing GAs and CKs, but increasing the concentration of ABA. It has been indicated that ABA content in roots and shoots of flooded pea plants (*Pisum sativum* L.) increases up to 3-8 fold (Zhang and Zhang, 1994). Reid and

Crozier (1971) has suggested that the reduced levels of GA₃ and CKs in the shoots of flooded tomato plants might be the result of their reduced export from roots to shoots.

The accumulation of ethylene in soils, as well as in roots, under water logging is well documented. Accumulation of ethylene in soils becomes increasingly important at O₂ concentrations in the soil atmosphere below 9% (Hunt *et al.*, 1981). Because of the much lower rates of diffusion of gases in water as compared with air, the water film around roots entraps ethylene in root tissue has a number of effects on root growth and morphology (Marschner, 1995), simultaneously triggering anatomical changes in the root tissue and the export of ethylene or its precursor 1-aminocyclopropane-1-carboxylate (ACC). It also acts as a root signal inducing epinastic responses to flooding in the leaves of herbaceous plants (Jackson, 1990; Wang and Arteca, 1992). Aquatic plants with slow root ethylene synthesis (e.g. rice) shows a positive responses to ethylene which decreases the level of ABA, enhances tissue sensitivity to gibberellins and stimulates growth of submerged internodes (Knaap *et al.*, 1996). Resistance of *Rumex palustris* plants to flooding is a consequence of both the rapid shoot elongation promoted by ethylene. Hypoxia and ethylene affect both the transport within a plant and perception of IAA, and lead to its accumulation at the stem base (Liu and Reid, 1992). IAA and ethylene act together to stimulate the enlargement of the cortical cell layer.