CHAPTER 2

Review of Literature

2.1 Origin and distribution of *C. alismatifolia*

The genus *Curcuma* is a member of the Zingiberaceae family which is composed of about 70 – 80 species of rhizomatous annual or perennial herbaceous (Purseglove, 1974). The species are naturally found in mixed deciduous tropical forests and tropical broad-leaved evergreen forests of the tropical and subtropical regions. The geographic distribution of the genus stretches from India to Thailand, Indo-China, Malaysia, Indonesia and finally to northern Australia (Apavatjrut *et al*., 1999). Thailand seems to be one of the richest areas for center of diversity of the genus *Curcuma* because about 50 species of the genus have been naturally recovered in Thailand (Larsen and Larsen, 2006). *Curcuma alismatifolia* Gagnep is the main cultivar that has gained popularity in the international market as the newcomer of cut flowers and ornamental plants. This species is native to Indo-China, comprising of Thailand, Laos and Myanmar (Gagnepain, 1908), common on the plateau of northeastern Thailand and the distribution range extends to the low land of coast of Thailand and west of Cambodia (Paisooksantivatana *et al*., 2001).

2.2 Morphology of *Curcuma alismatifolia*

Curcuma alismatifolia is a perennial plant. The morphology of curcuma is well documented. It is commercially propagated from subterranean organs, consisting of a rhizome or stubbed rhizome with several storage roots (Roh and Lawson, 1993; Hagiladi *et al*., 1997a). The subterranean organs reserve nutrients, moisture and food. They are used for supporting the shoot growth and development. The morphology of curcuma is described below.

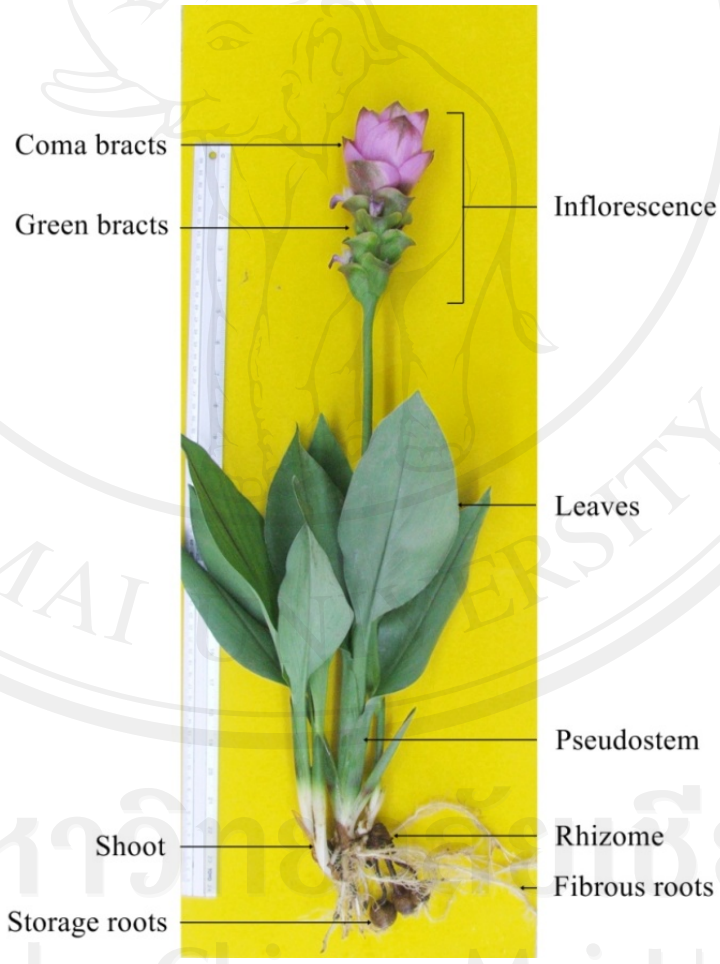

Figure 2.1 Morphology of *Curcuma alismatifolia* Gagnep.

2.2.1 Root system

Curcuma alismatifolia has two types of roots. The first type is a group of fibrous roots (Fig. 2.2A) that develop from storage roots and from the base of new shoot as soon as rhizome sprouts (Hagiladi *et al.*, 1997a). The second one is the contractile roots (Fig. 2.2B) which also initiates from the base of new shoot and develops into the new storage roots (egg-shaped root) when the growing season ends (Hagiladi *et al*., 1997a; Chidburee, 2008).

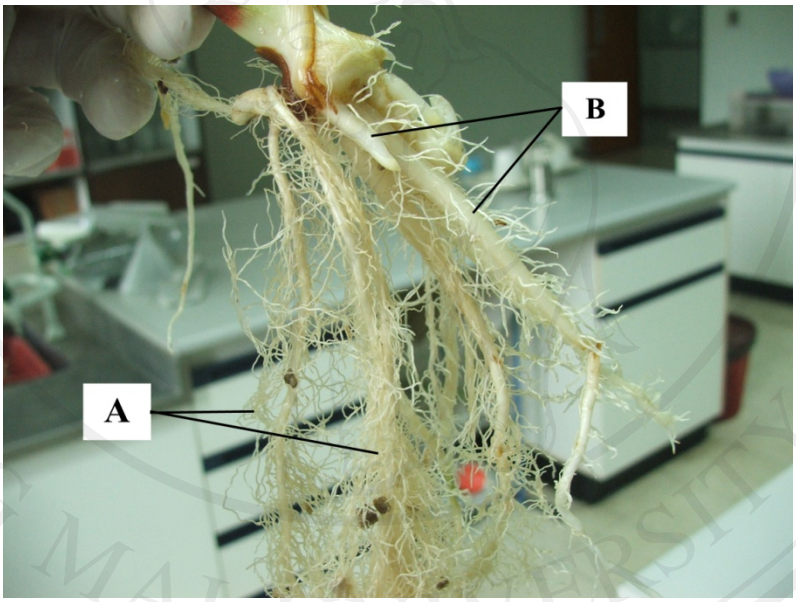

Figure 2.2 Fibrous roots (A) and contractile roots (B) of *C. alismatifolia*.

2.2.2 Underground parts

The underground parts of *Curcuma* plants comprise of a rhizome (Fig. 2.3A) and storage roots (Fig. 2.3B) which have different functions and both underground organs are connected by a thick contractile root (Fig. 2.3F).

Rhizome

A rhizome consists of buds, nodes and internodes (Fig. 2.3C, 2.3D and 2.3E). It is a specialized, modified form of the vertically-growing stem which short internodes (stubbed rhizome). Because its growth is more horizontal than vertical, thus it is called a tuberous rhizome, it is attached with 2 to 3 stolon-like storage organs termed “storage roots” (Phongpreecha, 1997). During senescence, the base of pseudostem swelled and develops to a new rhizome (Sukhvibul and Thongtaksin, 1995). During post-harvest, a rhizome with storage roots has prolonged keeping time than a rhizome without storage roots (Phubuopuen, 1992; Wannakrairoj, 1996), however, a rhizome without storage roots also does flower but very late compared with one which has storage roots (Hagiladi *et al*., 1997a).

Storage roots

Storage roots are modified thick contractile roots that connect with rhizome. The end of contractile roots is swollen and becomes a round ball shape (Fig. 2.3B). The storage roots have high water content, food reserve, particularly carbohydrates (Ruamrungsri *et al*., 2001; Khuankaew *et al*., 2009). The number of storage roots is also thought to affect flowering time, inflorescence stem length and number of stems per rhizome (Phongpreecha, 1997). Thus, the number of storage roots can be used to determine the quality of a propagule or rhizome (Wannakrairoj, 1996).

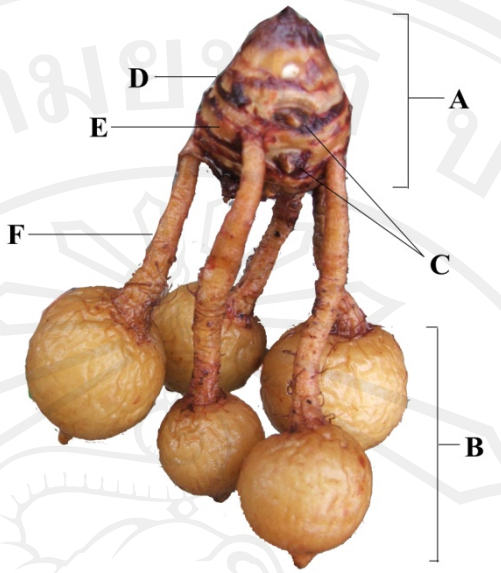

Figure 2.3 A rhizome with storage roots of *Curcuma alismatifolia*. : (A) rhizome, (B) storage roots, (C) buds, (D) nodes, (E) internodes and (F) connecting roots.

2.2.3 Aboveground parts

Pseudostem

Psuedostem comprises of basal foliage leaves (leaf sheath), tightly enfold together around inflorescent stalk (Fig. 2.4A).

Leaves

The leaves of *Curcuma alismatifolia* consist of leaf sheaths (Fig. 2.4C) that tightly enfold the pseudostem (Fig. 2.4A) and leaf blade (Fig. 2.4B). The foliage leaves are mostly elliptic (oval and flat in a plane, narrowed to each end which is rounded) with penni-parallel (veins run parallel for the length of the leaf, from the base to the apex), strongly ascending veins (Gerald, 1997). The leaf blade size is

about 4-5 cm width and 30-35 cm length, mostly deep green and median nerve is green or reddish (Fig. 2.4D) (Phubuopuen, 1992; Wannakrairoj, 1996). Leaves are oppositely arranged in a flat, two dimensional planes (Phongpreecha, 1997).

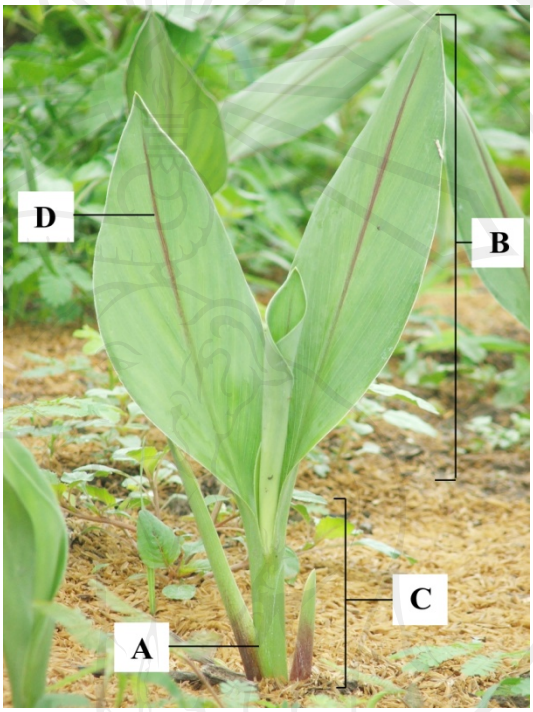

Figure 2.4 Foliage leaves of *Curcuma alismatifolia*. : (A) pseudostem, (B) leaf blade, (C) leaf sheath and (D) reddish vein.

Inflorescence

The inflorescence of *Curcuma alismatifolia* is compact spike which sprouts from pseudostem. The compact spike consists of upper bracts or coma bracts (pink bract) (Fig. 2.5A) and lower bracts (green bract) (Fig. 2.5B). About 8-10 bracts are located in lower part of spike which is short and green in color. The base of each green bract connects together to form an overlapping cup-like shape which can store water. The upper bract or coma bract has purplish pink with brownish green tip, 12-15 bracts are arranged to overlap like lotus flower (Sukhvibul and Thongtaksin, 1995).

Flower

The true flowers (a delicate purple labellum with yellow medium stripe) (Fig. 2.5C) are hidden in the axils of bract which do not flower at the same time. True flowers blossom from the first bract of the basal position to top position of the inflorescence. Floret is 4 cm long hidden in the axils of the lower green bracts (Phubuopuen, 1993).

True flower are a bisexual flower or perfect flower, strongly zygomorphic and often are associated with conspicuous floral bracts in a spike (Gerald, 1997). The perianth (the outer envelope of a flower, consisting of either the calyx or the corolla or both) is in two whorls consisting of three tubular calyxes and three lobed petaloid tubular corollas. The androecium (the male reproductive part of the flower) consists of 1 fertile stamen, a large opposing petaloid labellum (deep reddish purple) (Fig. 2.5D) representing 2 connate staminodia and two smaller flanking petaloid staminodia (Fig. 2.5E). The gynoecium (the female reproductive part of the flower) consists of a single compound pistil of 3 carpels, a single nestled in a channel of the filament (Fig. 2.5F) and anther of the fertile stamen and an inferior ovary with typically 3 locules, each containing numerous axile ovules (anatropous ovules) (Gerald, 1997).

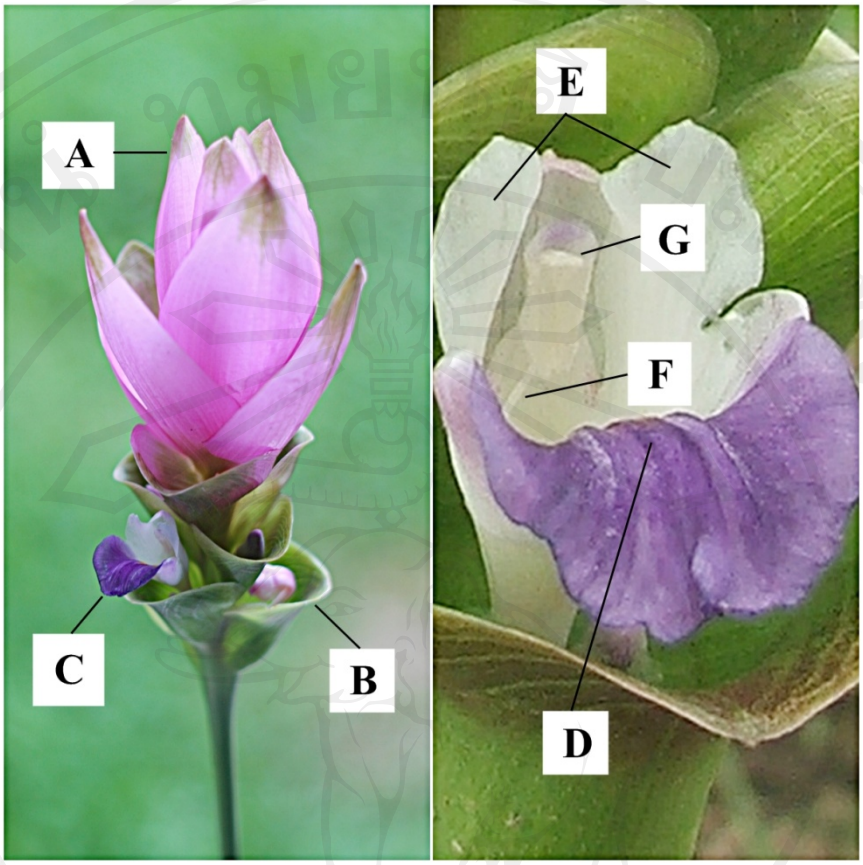

Figure 2.5 Inflorescence and true flower of *C. alismatifolia*.: (A) pink bract (coma bract), (B) green bract (lower bract), (C) true flower, (D) petaloid labellum, (E) petaloid staminodia, (F) filament and (G) anther cap.

2.3 Growth cycle of *C. alismatifolia* Gagnep.

C. alismatifolia Gagnep. is the perennial ornamental bulb that begins to be grown annually from March to September. Plants flowers during rainy season with 2-3 months of blooming period. It produces the inflorescence in 70 days after growing; thrusts the apex of floret in 90 days after planting; and as the first floret blooms, 105 days after growing, concomitantly the base of stalk begins to swell and contractile roots gradually stores water and food, develops into a knob at the end of the roots. Meanwhile, *C. alismatifolia* Gagnep. continually grows by producing averagely 2 – 5 rhizomes. However, during winter the plant is in its dormancy; the above-ground part of the plant is dried-up, so it's time for rhizome's harvest (Ruamrungsri *et al.*, 2005).

There are two types of planting dates; regular season planting (rainy season) and off-season planting (winter season). In regular season planting it can be divided into 3 periods, i.e., (1) early-season planting (February to March), (2) mid-season planting (April or May) and (3) late-season planting (June to July) (Wichailak, 2005). In off-season planting (winter season), the proposes are to: (1) avoid overage of the regular season cut flower or rhizome production, to avoid danger of epidemics and to increase income of growers, (2) satisfy customers at the time of their needs; and (3) guarantee employment throughout the year.

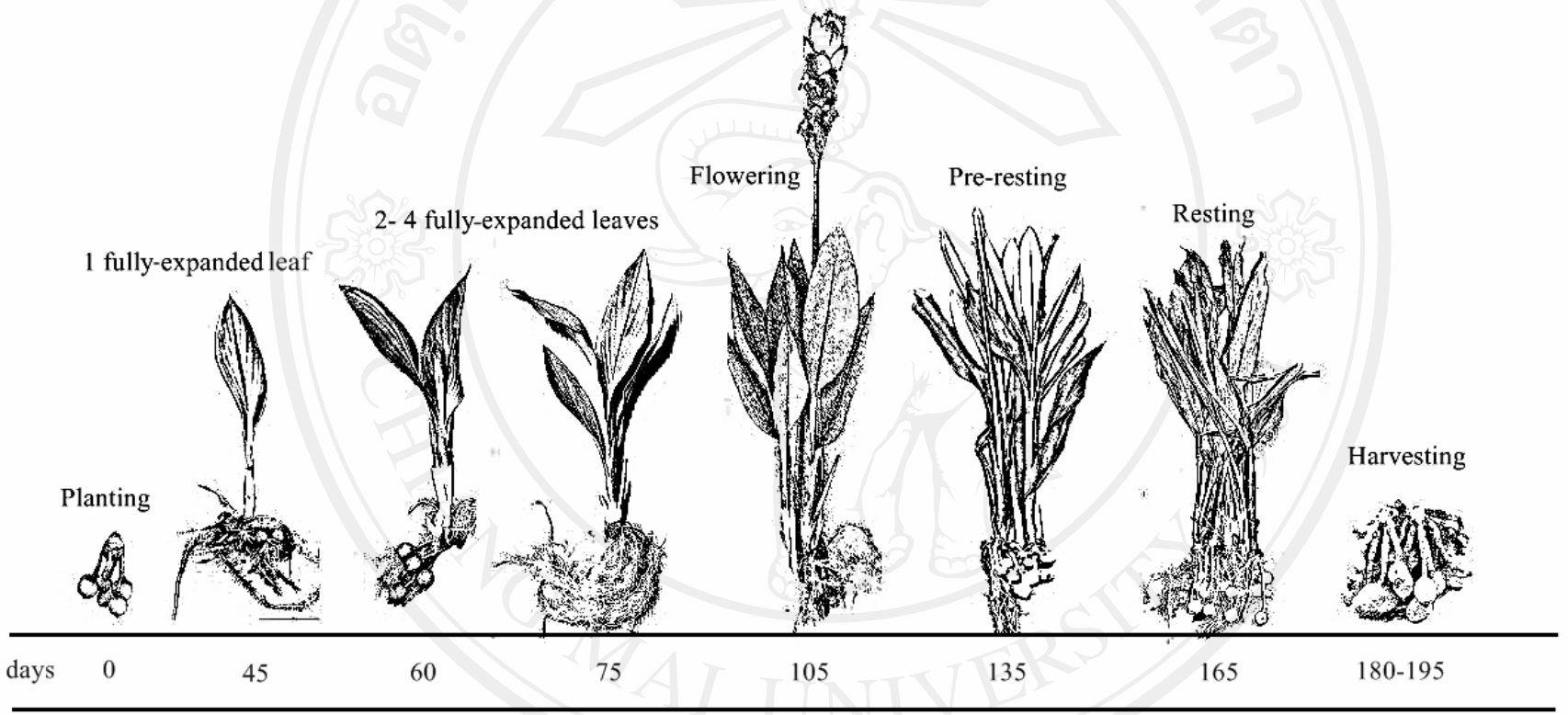

Figure 2.6 Growth of *C. alismatifolia* Gagnep.

2.4 Cultivation

2.4.1 Rhizome preparation

Use disease-free rhizomes, unaffected with nematodes and come from disease-free planting fields. The equal size of rhizomes should be selected for planting in the field. Generally, the rhizomes are graded according to sizes, as follows: large, with diameter larger than 1.5 cm; medium, with diameter range of 1.0 - 1.5 cm and small, with a diameter of less than 1.0 cm. Grade rhizomes and incubate in sand or rice husk charcoal or ground coconut husk medium, and the relative humidity should be maintained about 70% for stimulating uniform sprouting (Wichailak, 2005).

2.4.2 Planting area and soil preparation

Planting area should not be contaminated with rhizome rot or wilt disease and must be free of nematodes. In case of the land that is known to have been affected on curcuma should not be planted for at least two years. Land on which curcuma has never been planted previously should be selected. If land has been planted with curcuma, it should be rotated with other crops for at least three years before returning to curcuma again. The soil should be sandy loam with medium fertility, well-drained and having soil pH of 6.5-7.0. Avoid planting in the alkaline soil, by adding sulfur, as curcuma will be stunted and leaves become yellow with pale flower which is caused by the macro nutrient deficiency. Before planting, 15 g of 15-15-15 or 16-16-16 fertilizer (N: P_2O_5: K_2O) should applied in the bottom of planting hole and placed a rhizome by 7-10 cm deep (Wichailak, 2005).

There are two systems of planting curcuma in Thailand. The first is field planting: plows the land once and leaves it to be exposed to the sunlight for 20-30 days. As a preventive measure against rhizome rot disease, the urea mixed with lime at the ratio of 1:10 is applied to the soil at the rate 62,500 rhizomes per hectare before harrowing. The land is bedded, covered with plastic sheet and left for 15 days. The beds are sub-divided into small plots of about 400 m^2 and water drainage channels are provided. Within the sub-division, the beds are raised the level by 20-30 cm high and 1.0-1.2 m wide, provided a 0.5 m wide path for walking space and a 1 m wide between the sub divisions. The soil should not be plowed too deeply as the storage roots will grow down too deep, rhizome would get damaged when harvest and become undesirable for the market. The second is plastic bag planting: a potting material mixture (sand: rice husk or coconut coir dust: rice husk charcoal at a ratio of 1:1:1) is prepared and filled into a black plastic bag sized 15 x 30 cm, bags are then placed on a sheet of clear plastic on a raised planting platform of 20 cm high (Wichailak, 2005).

2.4.3 Planting preparation

In the field planting method, planting space depends on the sizes of the rhizome, i.e., large (30x30 cm or 62,500 rhizomes per hectare), medium (25x25 cm or 93,750 rhizomes per hectare) and small (20x20 cm or 125,000 rhizomes per hectare). For plastic bag planting: plant the sprouted rhizome close to the soil surface with upright shoot, this will result in inducing flowers about two weeks earlier than usual. After that, covers it with soil thinly in order to protect the shoot from burning (Wichailak, 2005).

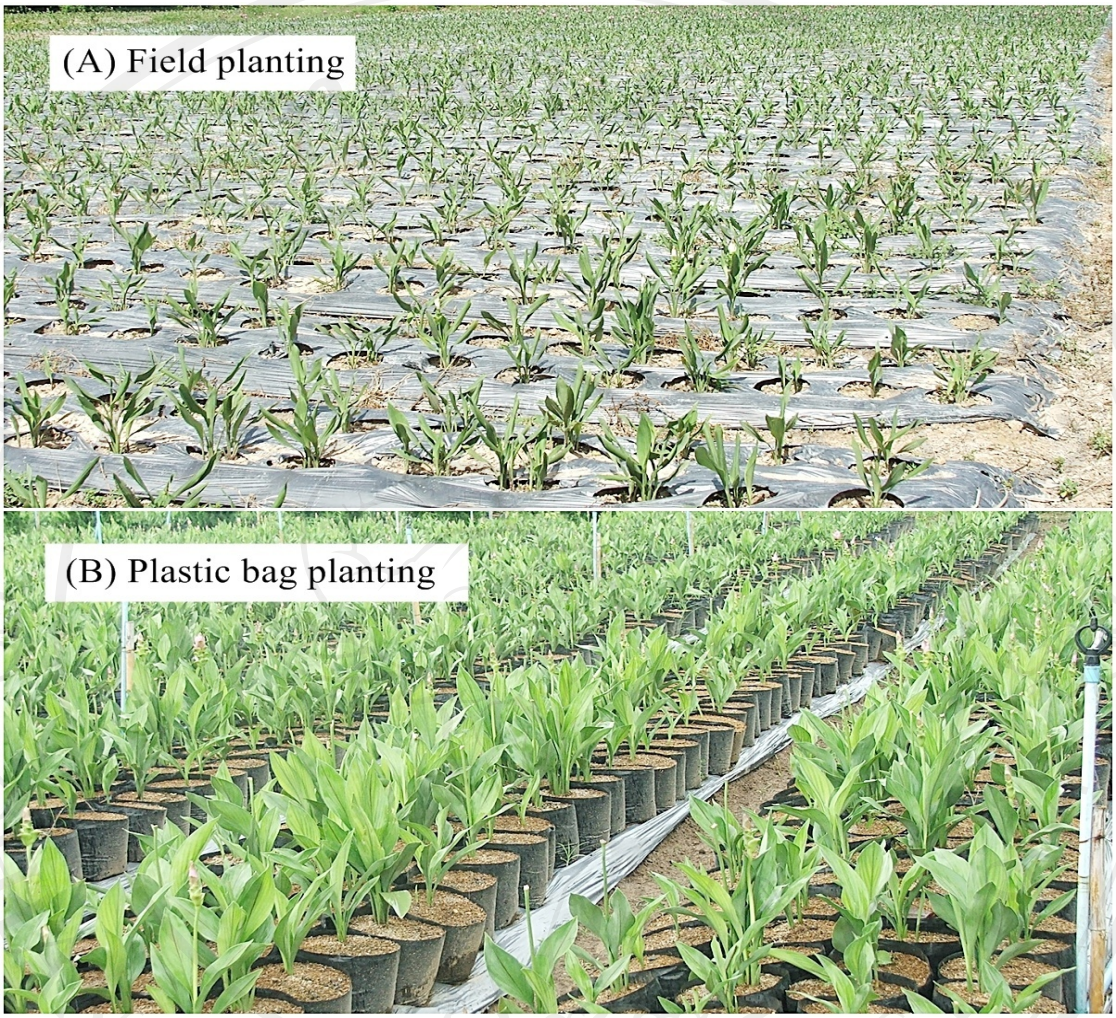

Figure 2.7 Planting of *Curcuma*: (A) field planting and (B) plastic bag planting.

2.4.4 Temperature and water

Curcuma plants originate in the tropical and subtropical areas, under high light intensity and consistent water supply. The water should be clean with pH in the range of 5.5-6.5, without contamination from any organic matters or toxic inorganic matters. High soil moisture is good for growth and development of curcuma, thus daily or often watering during curcuma growth and flowering are necessary (Wichailak, 2005).

2.4.5 Fertilizer application

For rhizome planting in the field, when two leaves unfolding occur, applying a high nitrogen fertilizer, such as 21-7-14, 15-0-0 or 16-16-16 (N-P_2O_5-K_2O) on rate 15 g per plant once a month. At flowering stage, 15 g of 13-13-21 (N-P_2O_5-K_2O) fertilizer should be applied per plant once a month. Furthermore, supplying foliar fertilizer (Ca, Mg, B, Zn, Cu) whenever plant shows the yellowing due to microelement deficiency. When new underground storage organ develop, giving high phosphorus and potassium fertilizers, such as 8-16-24, 14-14-21 or 13-13-21 (N-P_2O_5-K_2O) on rate 15 g per plant once a month (Wichailak, 2005).

For rhizome planting in a plastic bag, an amount of fertilizer application should be used at a low rate, about 7-10 g per plant, but more frequent every three weeks. Moreover, the completed nutrient culture solution can also be used for planting in a plastic bag in curcuma (Wichailak, 2005). The liquid fertilizer for planting curcuma in Thailand has been developed by H.M. the King's Initiative Center for Flower and Fruit Propagation, Chiang Mai University named "Banrai's Center or BC-1". The nutrient solution contains complete macro and micro elements by N 200, P 50, K 200, Mg 25, Ca 136, B 0.22, Mn 0.81, Zn 0.26, Cu 0.025, Mo 0.035 and Fe 0.41 mg L^{-1} (Ruamrungsri, 2005). Liquid fertilizer application supports the producing of new shoot and new rhizome quality.

2.4.6 Disease and pest control

The most damaging agent in *Curcuma* is bacterial pathogen which causes wilt and rot diseases. *Ralstonia solanacearum* is a causal bacteria, the symptom appears at the lower leaves which will roll due to the lack of water and it is clearly prominent in

the morning. The base of the plant and the new growth will appear succulent and leaf roll will spread to the upper parts, and then, the whole plant. The plant folds over, easily comes off the ground when pulled and finally, dries up or dies. The disease also causes rhizome and root parts to become succulent and transparent glassy appearance. It causes the rhizomes and roots to become dark and emits a rotting smell. The presence of nematodes will exacerbate the problem (Wichailak, 2005).

Nematode is an important factor in spreading the wilt disease and is commonly found in sandy soil. Control measure can be made by rotating crops that are not susceptible to the disease. Addition of organic matters, such as manure, fresh manure and humus, improves the physical properties of the soil, as well as, increase microorganisms that are antagonistic to nematodes. Collect nematode-infected rhizomes and roots from the planting ground and dispose by burying or burning (Wichailak, 2005).

Curcuma is infested by very few insects, except from the leaf roller. The grasshopper damages the leaves but can be controlled easily by synthetic pyrethroid, such as cypermethrin. Red spider mites cause spotting to the colorful bracts and can be controlled by dicofol with wetting agents.

2.4.7 Harvest and Post-harvest

For cut flower production: Plant watering should be done before harvest. The suitable time for cut flower is in the morning when 4-6 coma bracts open and 2-3 true flowers appear. Hold the base of inflorescence stalk then twist and pull up with one leaf attached. Soak in clean water and cover with a plastic bag. For preparation before transportation, keeping flower at low temperature 12-15˚C under a high

relative humidity at 85-95% about 1-2 hours before transport prolong flower quality. Before transportation, the basal end of inflorescence stalk should be wrapped in cotton wool and dipped in disinfectant solution (50-100 mg Clorox per liter of water). Pack it in a carton box and store in a 15-18 ˚C room (Wichailuk, 2005).

For rhizomes production: After the aboveground part of plant becomes senescent, the underground parts become dormant. The aboveground organs wilt and dry and rhizomes will be harvested. In case of planting curcuma in a field, the bed should be watered before harvesting rhizomes. Dig up rhizomes and wash out the soil, then separate rhizomes from a cluster. Cut the unhealthy rhizomes or storage roots by using the clean pruning shear (clean with 70% alcohol) and grade rhizomes according to rhizome size. Rhizomes will be immerged in the insecticide and fungicide solution and dried in the air under the shaded place for 14 days, then store rhizomes in well-ventilated place (shelf) (Wichailuk, 2005).

2.5 Background research

2.5.1 Nitrogen

Nitrogen is one of the major important limiting factors in agriculture production. The importance of nitrogen is that it is component of many important structural, genetic and metabolic compounds in plant cells. It is a major component of chlorophyll, amino acids, energy-transfer compounds such as ATP (adenosine triphosphate) and nucleic acids such as DNA (Ohyama, 2010). Soil nitrogen exists in three general forms; organic nitrogen compounds, ammonium (NH_4^+) ions and nitrate (NO_3^-) ions. Plants absorb nitrogen from the soil as both NH_4^+ and NO_3^- ions, but because nitrification is so pervasive in agricultural soils, most of the nitrogen is taken up as nitrate. Nitrate moves freely toward plant roots as they absorb water. Once inside the plant, NO_3^- is reduced to an NH_2 form and is assimilated to produce more complex compounds. Because plants require very large quantities of nitrogen, an extensive root system is essential to allow unrestricted uptake. Plants with roots restricted by compaction may show signs of nitrogen deficiency even when adequate nitrogen is present in the soil. Despite nitrogen being one of the most abundant elements on earth, nitrogen deficiency is probably the most common nutritional problem affecting plants worldwide. Healthy plants often contain 3- 4% nitrogen in their aboveground tissues. Major storage nitrogen in bulbs is protein-N which is assimilated from inorganic forms, both NH_4^+-N and NO_3^--N. Generally, protein-N fraction in higher plant amounts to 80 to 85% of the total N, the others are the nucleic acids-N (10%) and the soluble amino acid-N about 5% (Mengel and Kirkby, 1987).

2.5.2 Nitrogen utilization

2.5.2.1 Effect of nitrogen forms on N uptakes

Nitrogen is available in the biosphere primarily in three different forms: as molecular nitrogen (N_2), as mineral nitrogen (NO_3^- and NH_4^+) and as organic nitrogen (amino acids, peptides, etc.). The absorption of mineral nitrogen from the soil is one of the main functions of the plant roots. Uptake of mineral nitrogen (mainly NO_3^- and NH_4^+) occurs across the membrane in the epidermis, the outer most root cell layer, with the root and also in the root cortex cells (Lauter *et al*., 1995).

Nitrate (NO_3^-): Nitrate can be reduced to nitrite directly in the cytoplasm of root cells and, after nitrate is transported into plastids, it is further reduced to ammonium. The reduction is catalyzed by nitrate reductase (NR) and nitrite reductase (NiR), respectively. NO_3^- is a major source of N for plants. Compared with NH_4^+, NO_3^- has the advantage of being a storage form in plants with no necessity to be assimilated in the roots. In addition, NO_3^- nutrition induces an increase rather than the decrease in rhizosphere pH and there is no risk of toxicity at alkaline pH (Marschner, 1995).

Ammonium (NH_4^+): Most ammonium must be incorporated in organic compounds in roots, initially via the glutamine synthase/glutamate synthase pathway (Dennis and Emes, 1990). Ammonium does not normally accumulate in plant cells, but is assimilated into amino acids (Lauter *et al*., 1995). The assimilation of NH_4^+ in roots produces about 1 proton per molecule which has to be excreted into the external medium (Buchanan *et al*., 2002). Ammonium used as the sole source of N appears to have a negative effect on growth and morphogenesis. The negative effect of NH_4^+ has

been attributed to various factors such as changes in medium pH and toxic effects of free NH_4^+ (Raab and Terry, 1994, 1995; Walch *et al*., 2000)

Most plant species supplied with both NH_4^+ and NO_3^- forms can be more productive than those supplied with NH_4^+ or NO_3^- alone (Wang and Below., 1996). Maximum growth rates and plant yields could be obtained by combined supply of both NH_4^+ and NO_3^-. The preferential source has been shown to be dependent on plant species, cultivar and environmental factors. Strawberry is a plant which prefers NO_3^- (Darnell and Stutte, 2001) but total nitrogen uptake rate and growth was higher when both nitrogen sources were present in the solution (Ganmore-Neumann and Kafkafi, 1985). When both NH_4^+ and NO_3^- are supplied, pH stability may be achieved by similar rates of H^+ production (NH_4^+assimilation) and H^+ consumption (NO_3^- assimilation) and thus has a very low energy requirement (Raven, 1985; Allen *et al*., 1988). This may, at least, partly explain that optimal growth for most plant species is usually obtained with mixed supply of NH_4^+ and NO_3^-. However, when the NH_4^+-N/ NO_3^--N ratio was above 50:50, a yield decrease occurred in most aerobic crops with the exception of rice which, in most situations, grew better when the NH_4^+-N/ NO_3^--N ratio was above 50:50 (Smiciklas and Below, 1992). Many studies indicated that a reasonable NH_4^+-N/ NO_3^- -N ratio could increase crop yield and reduce NO_3^- content of vegetable (Dong *et al*., 2004; Chen *et al*., 2005). Moreover, different ratios of NH_4^+-N/ NO_3^--N in a nutrient solution or in soil with controlled nitrification not only affected plant growth, but also the organic acid content to a large extent. An increase in a suitable proportion of NH_4^+-N in nutrient solution led to a significant decrease in malate, citrate and fumarate (Dong *et al*., 2004). NO_3^- acts as the signal to initiate coordinated changes in carbon and N metabolism and organic acid production

(Scheible *et al*., 1997). During rapid vegetative growth, the rates of NO_3^- reduction, carboxylate and amino acid synthesis are fast. NO_3^- reduction implies the formation of excessive alkaline ions. These ions cannot be efficiently expelled from the cell, therefore, the plant synthesizes organic acids (principally citrate and malate) in leaves which are transported to the sites where NO_3^- reduction is occurring to maintain pH homeostasis (Touraine *et al*., 1988; Imsande and Touraine, 1994; Jose *et al*., 2000).

The ^{15}N assimilation during short time within 4.5 hours, *Curcuma* preferentially absorbed N from the combined NH_4^+ and NO_3^- supplied, followed by sole NH_4^+ and lowest absorption N in alone NO_3^- supply (Khuankaew *et al*., 2009). As similar in *Narcissus*, N was more absorbed when the combinations of both N forms (NH_4^+ and NO_3^-) were supplied (Ruamrungsri *et al*., 2000). However, it is different in tulip, the lower and upper half-roots absorbed N equally either from NO_3^- or NH_4^+, based on the root fresh weight (Komiyama *et al*., 2003). Nutrient medium containing both NO_3^- and NH_4^+ forms of N in proper combination are more suitable for growth of the cells compared to either form alone (Dubey and Pessarakli, 1994). The majority of the plant species grow better when N is supplied as a mixture of both NO_3^- and NH_4^+ forms in the soil. Certain plants can absorb either NH_4^+ or NO_3^- ions, depending on the pH of nutrient medium (Dubey and Pessarakli, 1994).

2.5.2.2 Role of amino acids

Plant nitrogen metabolism is regulated by nitrogen supply and by plant demand for growth. In recent years, it has become evident that many of the processes and metabolic pathways of plant nitrogen metabolism are regulated by the concentration of all or several amino acids and amides. The role of amino acids and amides in the regulation of nitrate and ammonium uptake, nitrate reduction, ammonium incorporation, protein metabolism and N remobilization is discussed. It is proposed that as the free amino-acid concentrations are dependent on the plant N status, changes in their cytoplasmic concentration may be involved in the regulation of plant growth and N uptake. (Barneix and Causin, 1996)

Khuankaew (2010) reported that the assimilated N in curcuma roots was mostly incorporated into asparagines (Asn). Asn is one of two amides (glutamine, Gln and asparagines, Asn) that play a central role in the nitrogen metabolism of plants (Bidwell, 1979) and it is often present in very high concentrations in both xylem and phloem sap to carry nitrogen away from source tissue (Lillo, 2004). Asparagine (Asn) is more soluble, less reactive and has nitrogen to carbon ratio higher than glutamine, all of which make it a better transport and storage compound (Lillo, 2004). Asparagine synthetase (AS) is considered as the major route for asparagines biosynthesis in plants (Lea *et al*. 1990). In an ATP - dependent reaction, AS catalyses the transfer of an amino group of glutamine to a molecule of aspartate (Asp) to generate a molecule of glutamate and asparagine (Coruzzi and Last, 2000). In other flower bulbs such as tulip and *Narcissus* roots, most of a major form of accumulated N is glutamine. In tulip, glutamine is a major form of the accumulated N in roots with

N supplied medium. On the other hand, the Gln content is very low and 4-methyleneglutamine (4-MeGln) is a predominant amino acid in the case of non supplying N (Ohyama, 1991). Ruamrungsri *et al*. (2000) reported that in *Narcissus* roots, most of the nitrogen in the 80% ethanol-soluble fraction was assimilated into glutamine.

2.5.2.3 Relation between nitrogen and cytokinin

Nitrogen and cytokinin (CK) content have been shown to be closely related (Horgan and Waering, 1980; Kuiper *et al*., 1989) but the molecular mechanism of interaction is just beginning to be understood. 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). 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 (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).

2.5.2.4 Effect of temperature on N uptakes and plant growth

Temperature are strongly related to nitrogen uptake, metabolism and assimilation, same as any other physiological and biological processes, and in some cases differentially affect NH_4^+ and NO_3^- uptake rates (Clarkson, 1985; Bloom, 1988; Engels and Marschner, 1995). Different plant species have different optimum temperature ranges for growth and yield. Optimum absorption and assimilation occur under normal temperature. The plants growth under lower- or higher-optimal temperature may affect on the rate of N uptakes, remobilization and assimilation, as well as, the assimilation of nutrients by influence on the assimilatory enzymes (Dubey and Pessarakli, 1994). In rice (*Oryza sativa* cv. Koshihikari), extreme high day temperatures during the grain-filling period may reduce starch synthesis in the grains, especially under N-deficient condition (Ito *et al*., 2009). High temperatures also induced an accumulation of sucrose and decrease in carbon and nitrogen transport from shoots to the ears via the phloem in rice (Ito *et al*., 2009). Low root temperatures reduce uptake rates generally, but may reduce NO_3^- uptake to a greater extent than NH_4^+ uptake (Clarkson and Warner, 1979; Macduff and Wild, 1989).

Temperature is the major factor that affects bulb growth. It is commonly used to hasten or delay bulb growth and development (De Hertogh and Le Nard, 1993). In

curcuma plants, temperature has effect on shoot sprouting, plant growth, flowering and rhizome dormancy. Shoot sprouting is enhanced under moisture and high temperature (30˚C) conditions (Hagiladi *et al*., 1997b). In their native habitat, the growth and flowering of curcuma in Thailand occurs during rainy season under average temperature at 25-30˚C, 13 hours photoperiod (long days) and about 80% relative humidity. After flowering, the plant becomes dormant when the temperature is low to 20 ˚C and short day length about 10 hours under dry season (Chidburee, 2008). In *C. alismatifolia*, vegetative growth and flowering is maintained by moisture and long day, and favored by a high temperature (Zhang *et al*., 1995; Hagiladi *et al*., 1997b; Changjeraja *et al*. 2007) and entering rest period is triggered by short days and/or lowering temperatures (Hagiladi *et al*., 1997b). *Curcuma* plants growth under 28˚C give the greatest development in aboveground parts, such as longest shoot and leaf length, greatest number of leaves, darkest of leaf color and highest dry weight of leaves, while at 18, 20 and 22˚C do not produce inflorescence and delay growth and development (Changjeraja *et al*., 2007). Its growth at low temperature (18˚C and 20˚C) give higher of total non-structural carbohydrate in leaves, rhizomes and storage roots. Moreover, the day and night temperature has effects on growth and flowering of this plant. Changjeraja *et al*., (2007) reported that high temperature 36/24˚C (day/night) increased vegetative growth of aboveground parts and flower but decreasing of night temperature to 24/18˚C supported dry weight accumulation of underground storage organs.

2.5.3 Critical nutrient level determination

Various models have been developed to describe the response of plants to nutrient supply and accumulation (Goodall and Gregory, 1947). Pfeiffer *et al*. (1942) proposed a hyperbolic model in which plants approached an asymptote or maximum value as nutrient accumulation increased. Linear models have been proposed to describe growth responses to nutrient accumulation (Goodall and Gregory, 1947). Other researchers identified a three-phase model (Macy, 1936; Bates, 1971; Ulrich, 1976) (Fig. 2.8). In this model, growth curves describe a deficient level of nutrient accumulation, region of poverty adjustment, or minimum percentage where yields rise with increasing internal concentrations of nitrogen. In the second zone of the growth curve, a transition from deficiency to sufficiency occurs, followed by a region known as ‘luxury consumption’ in which internal concentration of nitrogen rises but yield does not rise. The concentration of nitrogen at the transition from deficiency to sufficiency is known as the critical concentration. Eventually, nitrogen accumulation will rise to excessive or toxic levels.

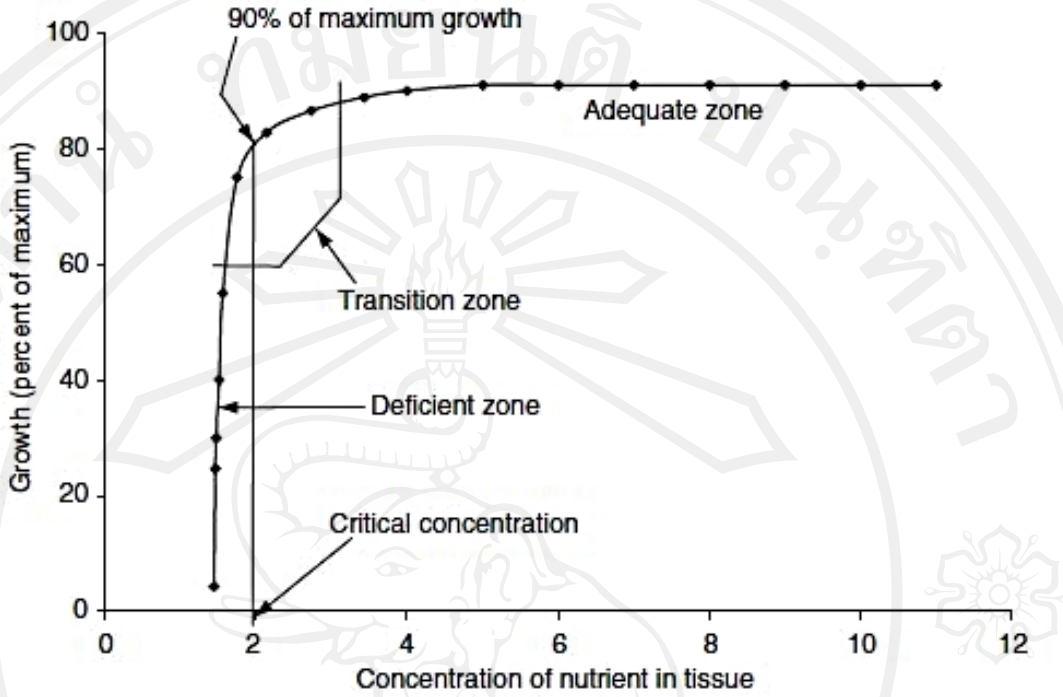

Figure 2.8 Model of plant growth response to concentration of nutrients in plant tissue. Units of concentration of nutrient in tissue are arbitrary. The model shows the critical concentration of nutrient at a response that is 90% of the maximum growth obtained by nutrient accumulation in the tissue. Deficient zone, transition zone and adequate zone indicate concentrations at which nutrients may be lacking, marginal or sufficient for crop yields (Source: Anonymous, 2009).

Critical nutrient levels are determined from the relationship between the nutrient concentration in selected plant tissue and plant growth and/or yield. In graphical form, this relationship is called 'the nutrient calibration curve'. In studies by Macy (1936), the nutrient calibration curve included the zones of minimum per central, poverty adjustment and luxury consumption. Macy (1936) proposed a central

concept stating that there is a critical percentage of each nutrient in each kind of plant, above which there is luxury consumption and below which there is poverty adjustment which is almost proportional to the deficiency until a minimum percentage is reached. The critical percentage is the nutrient concentration in the tissue at the interception of the luxury consumption and poverty adjustment portions of the nutrient calibration curve that is fitted by graphical procedures. Ulrich (1952) defined the critical nutrient concentration with respect to plant growth either in terms of the nutrient concentration that is just deficient for maximum growth or that which is just adequate for maximum growth or as the concentration separating the deficiency from the adequacy zones. The usual procedure is to draw the calibration curve free-hand to best fit the values from the respective X and Y coordinate. This derivation has two major criticisms, namely, the location of the curve through the point and the selection of growth just below maximum growth on the curve are arbitrary. Ulrich and Hills (1973) resolved the latter criticism by defining the critical nutrient concentration as the nutrient concentration in the tissue associate with a 10% reduction from maximum growth due to a deficiency.

Growth curve relating yield with tissue nutrient concentration are described in mathematical terms based upon hypotheses underlying the growth process. Mitscherlich was one of the first to establish a growth law model for plant species by quantifying the relationship between yield and nutrient supply in soils from field and pot experiments (Ware *et al*., 1982). The classical Mitscherlich equation that based on Liebig's Law of the Minimum (the response function is directly proportional to the amount of nutrient available in smallest supply) describes the response of a crop to an increase in the factor that is limiting growth, other factors being constant (Von Liebig,

1855; Mitscherlich, 1909, 1913). Although Mitscherlich equation was assumed to apply to all factors that could limit growth ('vegetation-factors', 'growth-factors' or 'production factors'), such as light, temperature, water and nutrients, it is most commonly applied to nutrients, in particular, nutrients supplied in the form of chemical fertilizers or organic soil amendments.

Mitscherlich plant growth model is based on a first-order rate equation and has been used to characterize the relationship between yield and nutrient supply in soils from field and pot experiments. The rate equation is given by

$$dy/dx = \alpha(\beta - y) \qquad [1]$$

where it is assumed that the rate of increase in yield is proportional to the decrement from the maximum yield obtained. In applying this model for determining plant tissue, critical nutrient values y denotes plant yield at a tissue nutrient concentration x, β the asymptotic maximum yield as x approaches infinity, $(\beta - y)$ the decrement from maximum yield, and α the constant of proportionality. The rate of increase in yield as a function of tissue nutrient concentration then follows Eq. [1].

Solving Eq. [1] with the initial condition that y equals zero at tissue nutrient concentration zero yields the Mitscherlich growth model written as

$$y = \beta\,(1 - e^{-\alpha x}) \qquad [2]$$

For plant tissue analysis in relating yield to tissue nutrient concentration, the assumption that at $x = 0$, $y = 0$ may be too restrictive. Therefore, under the initial assumption that at $x = 0$, there is some yield, $\mathbf{y_0}$ results in a three parameter model written as

$$y = \beta (1 - \gamma e^{-\alpha x}) \quad [3]$$

where $\gamma = (\beta - y_0)/\beta$. The parameters α, β, and γ of Eq. [3] are to be estimated from observed data.

To find the tissue nutrient concentration associated with 90% maximum yield let

$$y/\beta = 1-\gamma e^{-\alpha x} \quad [4]$$

where y/β = 0.9. The solution of Eq. [4] for x, the critical nutrient level, yields.

$$x = -\ln(0.1/\gamma)/\alpha \quad [5]$$

The determination of critical levels has been approached in several ways. A common definition of critical level in the literature is the tissue nutrient concentration corresponding to 90% maximum yield when only that nutrient limits growth (Ulrich, 1952). In a study of fish geranium (*Pelargonium hortorum* Bailey), the critical level was defined as the percentage dry weight of a mineral element in leaves at the onset of visual deficiency symptoms (Kofranek and Lunt, 1969). Nelson *et al*. (1979) defined critical level in a study of winter flowering begonia (*Begonia hiemalis* Fotch.) as the range between lowest leaf nutrient concentration without visible deficiency symptoms and highest leaf nutrient concentration with visible deficiency symptoms. Barraclough and Leigh (1993) determined critical levels for perennial ryegrass (*Lolium perenne* L.) to be near-maximum yield based on nutrient concentrations expressed on a fresh weight basis. Hershey and Paul (1981) reported potassium critical level in chrysanthemum (*Chrysanthemum morifolium* Ramat.) in three ways: the leaf potassium level corresponding to maximum yield, the level at 90% of

maximum yield and the level present in the first leaf with visible deficiency symptoms.

In many reports, the definition of critical level extends to soil solution and applied nutrient solution concentrations. For example, Timmer and Parton (1984) defined critical level as the soil solution nutrient concentration range associated with one standard deviation from the mean of maximum red pine (*Pinus resinosa* Ait.) seedling growth. Maust and Williamson (1994) compared relative yield in citrus (*Citrus* spp. L.) to six N treatments in order to determine an applied critical N level of 16.0 mg N/L. The relative yield was reported as an average of two experiments conducted at different growth stages. Applied critical N level data also exist in the literature for woody ornamentals. Japanese holly (*Ilex crenata* Thunb.) reportedly achieved a maximum rate of shoot dry weight gain when irrigated with a N concentration of 87 mg/L solution in one experiment (Niemiera and Wright, 1982a) and 100 mg/L in another (Niemiera and Wright, 1982b). Critical nutrient concentrations expressed on a fresh weight basis can be unreliable due to fluctuations in water availability and water loss during sampling (Barraclough and Leigh, 1993). Soil solution nutrient concentrations may yield inconsistent results due to variation in soil moisture, soil depth, soil organic and inorganic matter, temperature, rate of fertilizer application and pH of the soil solution (Marschner, 1995). The use of applied N for critical level determination also has limitations because it assumes that the applied N is present and available in the soil solution in quantities necessary to meet plant needs (Tanji and Stevenson, 1982).

2.5.4 Nitrogen status in *Curcuma*

In *Curcuma alismatifolia*, a major organ of nitrogen (N) storage is a stubbed rhizome (true stem) which accumulated 4-5% of N based on dry weight while only about 1-2% N is stored in the storage roots at the beginning of dormancy. Moreover, a major part of N in rhizomes and storage roots at this period is an insoluble N forms which 97% of N in rhizomes and 88% in storage roots. After the dormancy release, the portion of N in rhizome slightly decreases while increases in storage roots (Ruamrungsri *et al*., 2001). According to Tapun and Ruamrungsri (2006), N concentration in both mother rhizome and storage roots were changed during plant growth and development. Between planting to shoot growth and two leaves were fully expanded, the N concentration in mother rhizome increased due to being derived from storage roots and some N were transported to shoot. At flowering, the N in mother rhizomes and storage roots decreased whereas the most of N were accumulated in leaves and some in inflorescence. During this stage, the new underground storage organ started to reserve food and at the rest period, under dry and low temperature, N concentration increased in both new rhizome and new storage root.

Ruamrungsri and Apavatjrut (2003) reported that N played an important role in the growth and quality of curcuma rhizomes and flowers. A high level of N supply enhanced the number of flowers and rhizomes, resulting in enhanced plant growth but new storage roots were depressed. The formation of storage roots might be influenced by more complex nutrient conditions, such as the carbon and nitrogen balance. A high level of the N application increased free amino acid concentration in rhizomes and storage roots (Ohtake *et al*., 2006).

Nitrogen deficiency in curcuma results in stunt growth and decreased the leaf area, leaf yellowing and reduced flower quality. When curcuma plants were cultured in N-free solution, the number of new shoot and leaves per plants was much reduced compared with culture in complete solution (Ruamrungsri *et al*., 2006). Ohtake *et al*. (2006) reported that lack of N supply decreased total N concentration, total amino acids and protein concentrations in both rhizome and storage root. On the other hand, starch and sugar concentration increased in the rhizomes and storage roots when plants were cultivated in N-free culture solution.