Chapter 7

General discussion and conclusion

7.1 General discussion

7.1.1 Physiological mechanism of rhizome formation under natural environmental and *in vitro* conditions.

Physiological mechanism study during rhizome formation was carried out both under natural light condition in open field and *in vitro*. The vegetative growth related to cell enlargement and translocation of assimilates from source (leaves) to sink (rhizome and storage roots) were determined.

Rhizome formation study under natural light condition was done in 2004. Mother rhizome with 2.5 cm and 4-5 storage roots were grown in plastic bag using sand : rice hull : rice husk charcoal at the ratio of 1 : 1 : 1 as planting media. Vegetative growth, cells number and cell width of rhizome were measure every week. Accumulations of carbohydrate were determined in leaves, new rhizome and storage roots every week, to investigate the translocation of food reserve from leaves to storage organs in underground part.

The result showed that morphogenetic events of rhizome formation of *C. alismatifolia* could be separated into 3 stages i.e. 1) induction and initiation stage II) differentiation and development stage and III) ripening stage. Stage I, the induction period was estimated at 11-13 WAP, appearance by the decrease of vegetative growth and carbohydrate in leaves. Then, initiation occurred at about 15 WAP when assimilates were translocated to rhizome and storage roots. Differentiation and development occurred from 15 WAP to 22 WAP when cell division and expansion actively occurred. Rhizome ripened at about 23 WAP when the growth of rhizome and storage roots were maximum. The enlargement of rhizome and storage roots appeared at the same period as TNC accumulation (from 15 WAP) when the sunshine duration was about 12.5 hr in late August. Although, the partitioning of assimilates

(TNC) from leaves to new rhizome and storage roots gradually started from the first stage of growth (2 WAP), it was actively increased from 15 WAP. The increase of TNC occurred to be synchrony with cell enlargement (increase of cell width). Indicated that the translocation of carbohydrates was stimulated by the environmental condition from late August to the end of September when the sunshine duration was about 11.5-12.5 hr/day. Therefore, day length at 12.5 hr/day may be a critical signal to translocate storage reserves from source(leaves) to sink (rhizome and storage roots). Ruamrungsri *et al*. (2004) reported that the light interruption by 2 hr of night break treatment could inhibited storage roots formation of this plant, although it was in winter. The partitioning of assimilates to storage organ caused by interaction between light, darkness and circadian rhythm of photosynthesis process and sucrose biosynthesis which appeared to mediated by phytochrome (Thomas and Vince-Prue, 1997).

Since microrhizomes have got enough potential to be used by commercial growers and disease-free planting material, thus the research was further studied on rhizome formation *in vitro*. Shoot explants were cultured in different sucrose concentrations i.e. 3, 4, 5 and 6% in MS medium at pH 5.8. Growing condition was set at 22±2^{o}C, 16 hr light/ 8 hr of dark cycles, for 6 months. Then, changed the condition to induce rhizome formation for more 2 months. The results found that rhizome formation *in vitro* of *C. alsimatifolia* was affected by sucrose concentration in medium. The high concentration of sucrose at 6% was toxic and caused browning on tissue. Phenolic oxidation in browning tissue caused by the light mediated oxidation of polyphenols to quinines which are highly toxic to the culture tissues (Juma *et al*., 1994).

In addition, sucrose play an important role of carbohydrate source for rhizome formation and the optimum of sucrose level affected rhizome development (Chirangini and Sharma, 2005). However, the requirement of sucrose level depend on plant species, such as 6% for *Gladiolus* ‘Friendship’, 6-8% for *C. longa* (Dantu and Bhojuani, 1995; Sunitibala *et al*., 2001). In this experiment, the optimum of sucrose for rhizome formation was 4%. However, terminal of contractile roots did not swollen as in natural condition (Fig 4.2 c). This means that not only sucrose concentration but

also other factors involved the translocation of assimilates to form storage roots, which should be day length and/or temperature.

7.1.2 Effect of day length and red light on growth and rhizome formation

7.1.2.1 Day length

Many factors affected growth and rhizome formation of *C. alismatifolia* i.e. light, temperature, humidity and plant hormones. In chapter 5, effect of day length at 7, 10 and 13 hr were studied compared with under natural light condition. Plants were grown in growth room and supplied with 7, 10 and 13 hr of cool white fluorescent tubes (405-812 nm), 60 µmol $m^{-2}s^{-1}$ PAR at 27±2°C, 70-80% RH until harvest. The results indicated that day length did not affect floral initiation since floral bud was initiated in all treatments. However, short day at 7 and 10 hr inhibited floral development and flowering of plant. Ruamrungsri *et al.* (2005) found that under short day length in winter, extended day length using night break treatment by supplying 2 hr of artificial light source could promote flowering percentage and flower quality.

For rhizome formation, control of the formation by photoperiod may be either quantitative or qualitative response. Tuberisation of potato appears always to be hastened by short day, and depend on cultivars. Perception of day length is accomplished by leaves and one or more stimuli are then translocated to the responsive region (Thomas and Vince-Prue, 1997). The response of *C. alismatifolia* to day length was showed in experiment 5. Day length did not affect the number of new rhizomes per plant and the number of storage roots at dormancy. This means that rhizome yield do not change when plant grown under different day length. However, the number of rhizomes in control treatment was higher than the other day length treatments which it may be affected by different light intensity among treatments.

The result of photosynthesis rate indicated that it was fluctuation of photosynthetic rate in control during 3-5 WAP while they were gradually increased in 7 and 10 hr treatments at 5 WAP. Chlorophyll fluorescence of control was lower than the others indicated that there was some factors affected PSII efficiency of leave in control during this period. The explanation of photosynthesis behavior affected by day length still do not clear due to the short period of photosynthetic rate and chlorophyll

fluorescence measurement in this experiment. In *Begonia*, maximum tuberlisation required a daily photoperiod of at least 5 hr. The required response appears to be for light rather than carbon fixation (Thomas and Vince-Prue, 1997).

The decrease of total chlorophyll concentration, chlorophyll a and b in short day length (7 hr) may affected photosynthesis process and leaded to the lower concentration of assimilates such as TNC, Starch, TSS, RS and free sugars (fructose, glucose and sucrose). The effect of day length on partitioning of assimilates between storage organs cause by the interaction between light, darkness and circadian rhythms in component process of photosynthesis and sucrose biosynthesis. Phasing of the rhythms by light appears to be mediated by phytochrome. However, the pattern of assimilate partitioning influence by day length is not consistent when plant grown under long term period (Thomas and Vince-Prue, 1997).

Not only carbohydrate concentration but also mineral nutrients in rhizome and storage roots were reduced by short day length. Similar to in chrysanthemum, long day treatments increased N, K, Ca, Mg and carbohydrate in leaf (Sharmugam and Muthuswamy, 1974) and *Medicago sativa* which N uptake and partitioning to tap root were reduced with short day duration.

Gene expression is usually quantified by measuring the steady-stat level of gene products (mRNA or protein) (Verhees *et. al*, 2002). This technique benefits gene expression analysis in relation to plant development can be studied in individual samples in relation to the morphological changes like during rhizome formation. Photoperiod control several responses throughout the plant life cycle, like germination, flowering, tuber or rhizome formation, onset of bud dormancy, leaf abscission, and cambium activity (Martinez-Garcia, 2002). From light-regulated responses as report in this research, the significant latency period before and effect become apparent is the action of gene. It was found that three of polymorphic bands were differential expressed in rhizome of plant grown under different day length. Hart (1988) revealed that the light may regulated gene expression assigned the point of possible photocontrol to the level of transcription. In potato, using RT-PCR differential display of leaves from plants grown under inductive (short day) and non-inductive (short day and night break) condition have induced for tuberization (Amador *et al*., 2001).

7.1.2.2 Red light

In some species, not only day length but also red light regulated tuberization. In *Begonia*, 1 min of red light at 8 W m^{-2} completely prevented tuber formation (Thomas and Vince-Prue, 1997). Effect of red light on growth and rhizome formation of *C. alismatifolia* was studied. Plant were grown under red light (632-660 nm), cool day light (405-812 nm) compared with natural light source. The condition of growth room was set up at 22±2°C, 70-80% RH. In *C. alismatifolia*, red light did not inhibit rhizome formation but it reduced quality of rhizome by decreased diameter of rhizome and storage roots. Life cycle of plant also was hastened by red light. Food reserves i.e. TNC and starch in rhizome and storage roots also were reduced by red light. This may be involved to enzyme activity for starch degradation. Photoregulated enzyme can be found in starch degradation and the effects of light on an enzyme are often described as photomodulatory (Hart, 1988).

Plant reached to dormancy stage at 19 WAP earlier than those grown under cool day light and natural light at 23 and 25 WAP, respectively.

Leaves area of plant under red light was lower than natural condition. It was found that the larger leaves area gives higher photosynthesis (Nivut, 1992). Because the leaves area of control was higher than those of red light and cool day light, it brought about by the higher photosynthetic rate. Kim *et al*.(2003) revealed that net photosynthetic rate of *in vitro* chrysanthemum was highest under red/blue LEDs followed by florescence and lowest under blue and far-red LEDs and blue light. In *C. alismatifolia*, red light reduced photosynthetic rate compared with control.

Total nitrogen concentration in new rhizome and storage roots of *C. alismatifolia* decreased when plant grown under red light. Similar to tobacco, which red light decrease chlorophyll content, leaf colour paler and increase invertase activity, reduce sugar content and lower N metabolism compared with blue light (HongZhi *et al*., 1998).

The result of gene expression showed the difference of four polymorphic bands in rhizome during grown under red light different with natural light (Fig. 6.10). Many plant genes, which encode proteins involved in photosynthesis or biosynthetic enzymes in secondary metabolism, have been found to change their expression patterns in response to light (Terzaghi and Cashomore, 1995). Kato-Noguchi (2004)

reported that the growth habit of Progress No.9 in red light is unlikely to be due to a light-induced blockage in transcription of the GA3 *β*–hydroxylase gene in *Pisum sativum*. The chlorophyll-associated proteins were inhibited in red light grown non-green plants (Sood *et al*., 2004).

7.2 Conclusion

Growth cycle of *C. alismatifolia* took place from August to November for 23 WAP. Process of rhizome formation could be separated into 3 stages i.e. 1) Induction and initiation stage 2) differentiation and development and 3) ripen stage. Induction stage occurred at 11-13 WAP when vegetative growth was maximal. Cell division and enlargement actively increased during 14-22 WAP socalled differation and development stage. Rhizome ripped at 23 WAP when its growth was terminated. Storage roots modified from contractile roots at 14-22 WAP (Aug to Oct). Translocation of TNC from source (aboveground parts) to sink (underground parts) started at 11 WAP and reached a maximum at 18 WAP. Then rhizome was harvested at 23 WAP.

The rhizome formation under field experiment indicated that the swollen of storage roots occurred during short sunshine duration in winter. This means that not only sucrose concentration but also other factors involved the translocation and accumulation of carbohydrate to storage organ. Therefore, the optimum condition should be studied further to stimulate the storing of food reserve in storage roots.

It was concluded that short day (7 hr) decreased the number of shoots and rhizomes per plant and number of storage roots per rhizome under sufficient light intensity in this experiment. Under low light intensity all the plants exhibited succulent growth, and produced only one shoot with one new rhizome. Short day treatment also decreased food reserve i.e. reducing sugar and K in new rhizomes and total nonstructural carbohydrate, reducing sugar, P and Ca in storage roots. Long day length is required for the rhizome and flower production of this plant.

C. alismatifolia. grown under red and cool day lights light intensity caused abnormal elongation of plants. These conditions also accelerated flowering, decreased photosynthetic rate and chlorophyll contents but increased chlorophyll fluorescence.

Illumination of plants during the entire experiment with red or cool day light at 60 µmol $m^{-2}s^{-1}$ PAR decreased yield and quality of rhizomes. Red light reduced the accumulation of TNC, starch and reducing sugar in the rhizome and storage roots. Fructose, glucose and sucrose in new rhizome and storage roots were lower in red light compared with cool daylight and natural light condition. Red light was also inhibited nitrogen accumulation in rhizome and storage roots. Differential of gene expression was found by a total of four polymorphic bans when plants grown in different light spectrum.

7.3 Suggestions for future experiment

The result from this research could indicate the effect of day length and red light on growth of rhizome. However, the relative factors involving the growth and development of this plant should be studied further. Moreover, effect of light intensity on rhizome formation should be done. This information should be very beneficial for a better understanding on growth and development of this plant.