Chapter 3

Rhizome formation under natural conditions

3.1 Introduction

In 1976, *C. alismatifolia* Gagnep was selected by Thai researchers of Chiang Mai University and the selected clone was called 'Chiang Mai Pink' with the attractive long lasting coma bracts and introduced to the market thereafter. In 2004, 75% of *C. alismatifolia* rhizome productions in Thailand (more than three million rhizomes) were exported to Japan, the Netherlands, USA and Australia (Department of Agricultural Extension, 2005). The size of rhizome affects growth and flower qualities. Therefore standard size of rhizome for export was set at about 2 cm in diameter with at least 4-5 storage roots (Department of Agricultural Extension, 2005). In Thailand, seasonal production starts in May by planting dry stored rhizomes. The flowering occurs from July to August during the rainy season in Thailand when the climatic conditions are 27-28^{o}C, 12-13 hrs of day length and 70-80% RH. Then the plants become dormant from November to December, when the daily sunshine duration is about 10 hrs. Rhizomes are usually harvested in December or January and are stored at room temperature with good air-ventilation. Reserve carbohydrate (total nonstructural carbohydrate (TNC), starch and soluble sugars) is important for rhizome survival and growth in the next season.

In this experiment, the research was carried out to study growth cycle, rhizome formation and the dynamic changes of TNC in source (leaves) and sink (rhizome and storage roots) before dormancy, was analyzed under natural environment.

3.2 Materials and methods

3.2.1 Plant materials preparation

Rhizomes of *C. alismatifolia* cv. Chiang Mai Pink with the diameter of 2.5 cm and 4-5 storage roots were grown in black plastic pots containing sand: rice husk:

rice husk charcoal at the ratio of 1:1:1 under natural environment (Fig. 3.1a-b). Shoots and roots emerged about 2 weeks after planting (WAP). Each plant was supplied three times a week with 100 ml of nutrient solution comprising 200 mg N, 50 mg P, 200 mg K, 65 mg Ca, 20 mg Mg, 0.22 mg B, 0.54 mg Mn, 0.26 mg Zn, 0.04 mg Mo and 0.45 mg Fe per liter and the solution was supplied three times a week. Two plants were sampled every two weeks, started from 3 WAP until 20 WAP at harvest.

a b

Figure 3.1 The rhizomes (a) and plantlets (b) of *C. alismatifolia* growing at Lampang province, Thailand.

3.2.2 Data collection

3.2.2.1 Measurement of vegetative growth

The growth and development in terms of plant height (cm), diameter of pseudostem (cm), number of leaves per plant, number of new rhizome per cluster, number of storage roots per rhizome, and dry weight of rhizome and storage roots (g) were measured.

3.2.2.2 Growth of cells and tissue development of rhizomes and storage roots.

Growth of cells and tissue during rhizome formation was observed using microtecnique (Johansen, 1940: see appendix A). The cell width and cell number of storage roots and contractile roots were measured.

3.2.2.3 Analysis of carbohydrate concentration in rhizomes and storage roots

Analysis of total non-structural carbohydrate by Nelson method (Hodge and Hofreiter, 1962: see appendix B) was done every two weeks to investigate the translocation of carbohydrates from source to sink during rhizome formation.

3.2.2.4 Meteorological data collection

The meteorological data (temperature, humidity and sunshine duration) were collected at Lampang Agriculture Research and Training Center, Rajamangala University of Technology Lanna, Lampang province. The figure 3.2 shows the meteorological data in 2004.

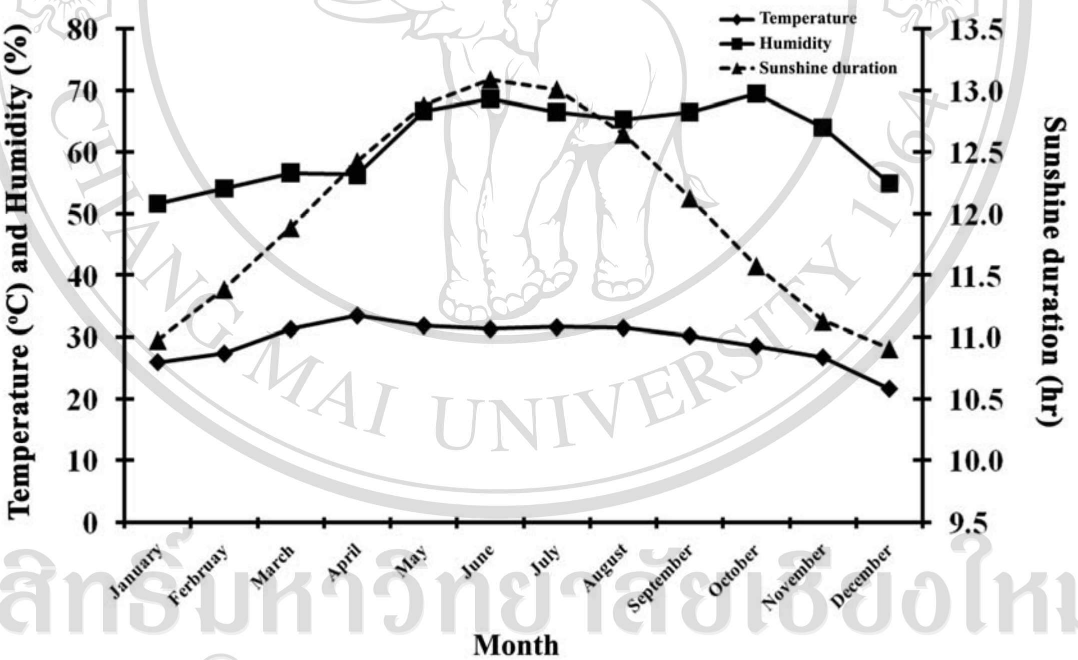

Figure 3.2 Average monthly temperature, relative humidity and sunshine duration in 2004 at Lampang province, Thailand.

3.3 Results

3.3.1 Growth of aerial parts (pseudo-stem and flowers)

Aerial parts comprised of foliage leaves, where the base of leaves wrap together around the flower stem so called "pseudo-stem". Plant grew relatively fast from 7 weeks after planting (WAP) to 11 WAP. The average height of the plants was about 42.40 cm. Then the growth was decreases (Fig 3.3a). The number of foliage leaves per plant also reached a maximum of 4 leaves per plant at 11 WAP (Fig 3.3b). The diameter of pseudo-stem gradually enlarged until 11 WAP to about 1.36 cm (Fig 3.3c). The 2^{nd} lateral pseudo-stem emerged at 7 WAP (Fig 3.5a-b) and the 3^{rd} and 4^{th} orders started from 11 and 15 WAP, respectively (Fig 3.3d).

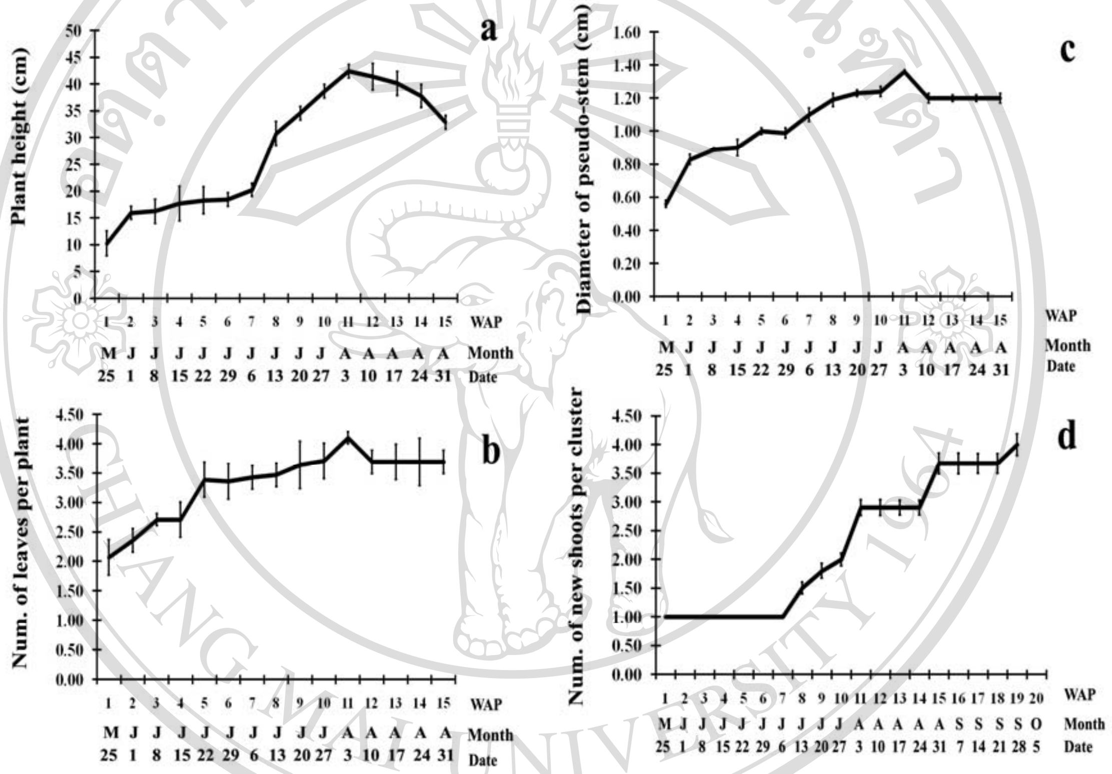

Figure 3.3 Characteristics of plants grown under natural environmental conditions.
a: plant height, b: number of leaves per plant, c: diameter of pseudo-stem, d: number of new shoots per cluster.
Data were means with standard deviations of ten independent measurements.

3.3.2 Changes of dry weight

Leaf dry matter dramatically increased from 5 WAP (Fig. 3.4a) and reached a maximum of 48.62 g within 11 WAP and rapidly decreased thereafter (Fig. 3.4a). Dry matter of new rhizome and storage roots linearly increased from 5 WAP to a maximum at 11 WAP (Fig. 3.4b-c). The dry weight of new rhizome was 7.32% higher than that of the mother rhizome at planting stage.

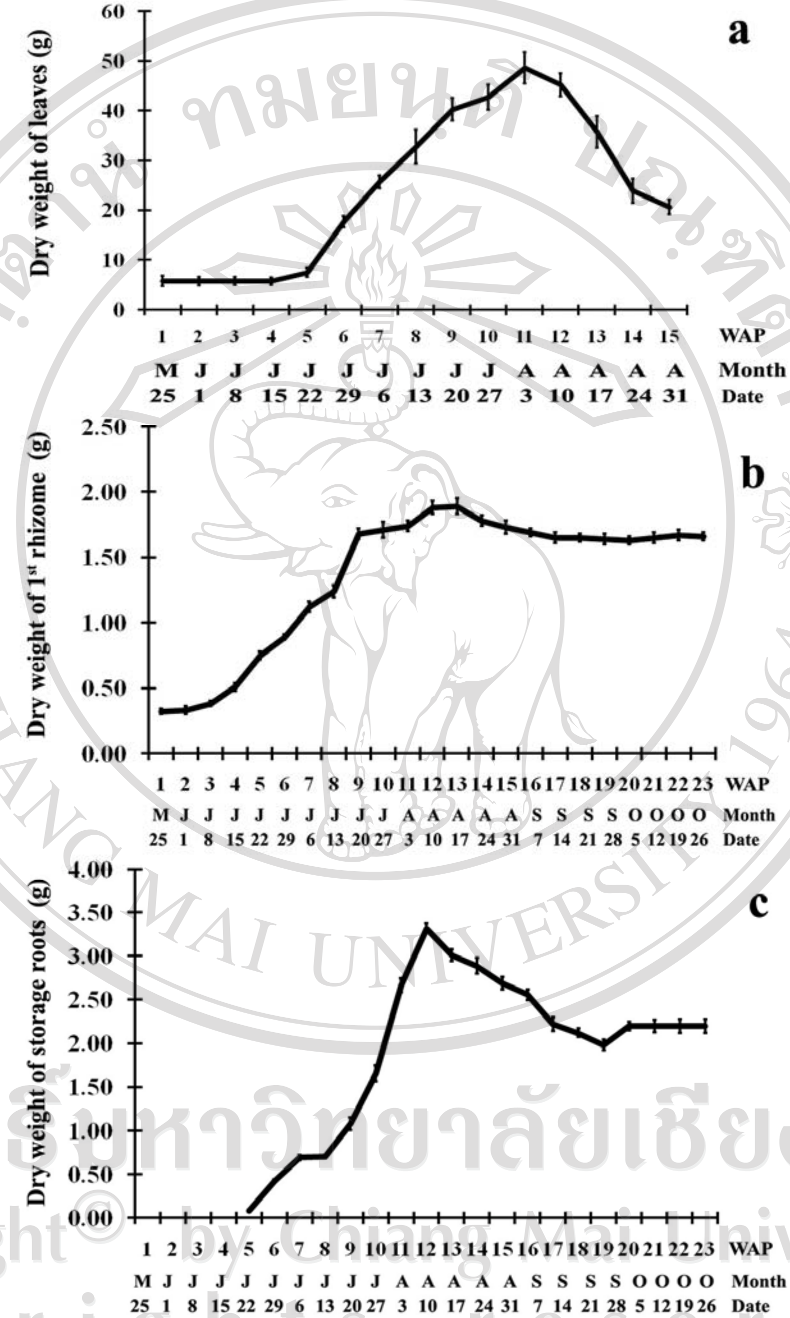

Figure 3.4 Dry weight of leaves (a), 1st rhizome (b) and storage roots (c) grown under natural environmental conditions.

Data were means with standard deviations of ten independent measurements.

a b

Figure 3.5 The 2nd lateral pseudo-stem emerged at 6 WAP (a) and 7 WAP (b).

3.3.3 Pattern of growth

The growth pattern of *C. alismatifolia* in Fig 3.6 showed that the 1st pseudo-stem (PS) emerged from the old rhizome in May at 2 - 3 WAP (Fig 3.6a). The 1st PS produced a few of contractile roots in June (Fig 3.6b). Branching of the 2nd and the 3rd PS emerged from lateral buds of new rhizome (Fig 3.7) after flowering in July (Fig 3.6c) and they flowered in August (Fig 3.6d).

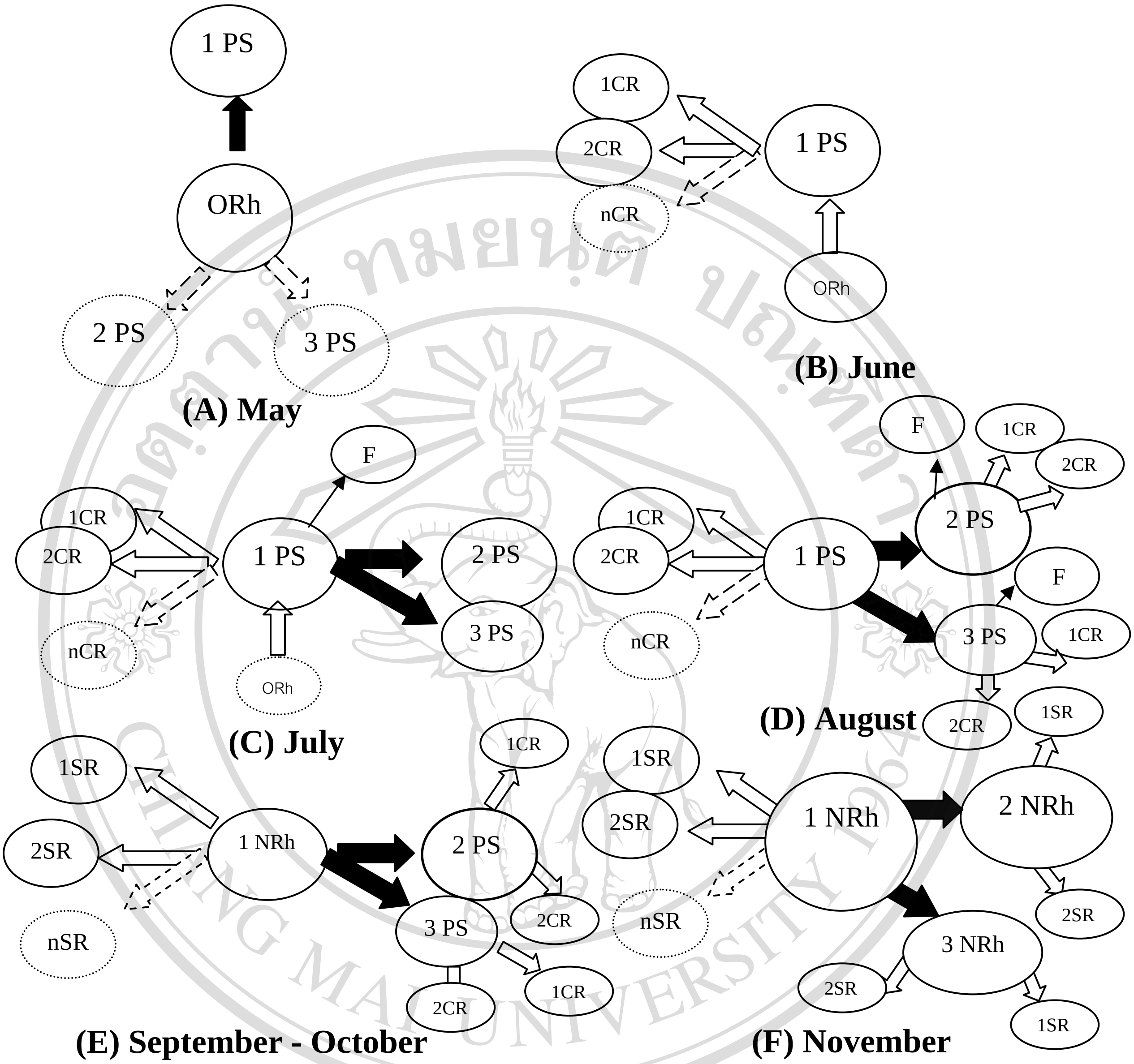

Figure 3.6 Flow diagram illustrating the development of *C. alismatifolia*.

ORh = old rhizome, PS = pseudo-stem, F = flowering, CR = contractile root, NRh = new rhizome and SR = storage root. Size of the circles indicated the relative size of the plant compartment. Arrows indicated the direction of allocation. Thickness of the arrows indicated the relative strength of the allocation.

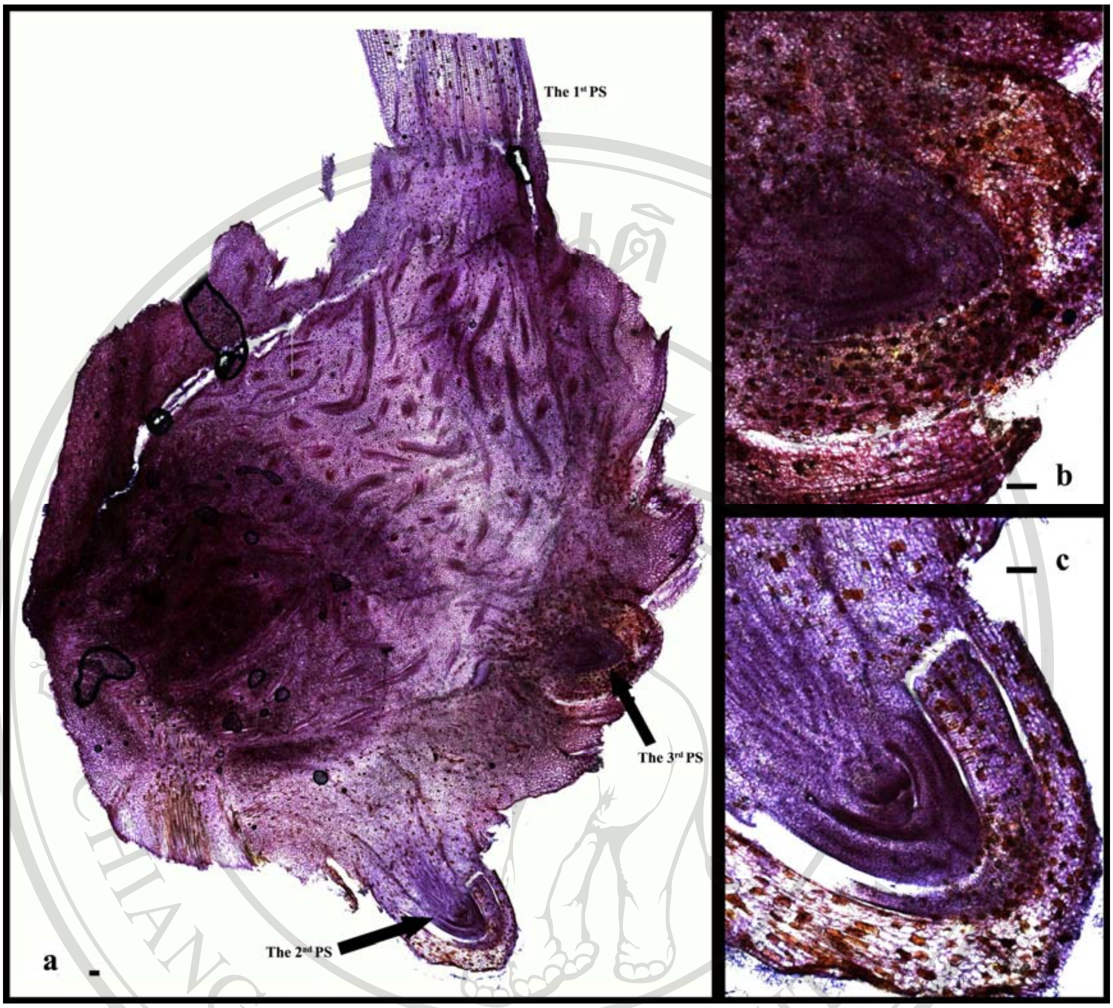

Figure 3.7 Longitudinal section of rhizome (a) (1st PS = first pseudo-stem and 2nd PS = second pseudo-stem (b) and 3rd PS = third pseudo-stem(c)). Bar = 200 µm

3.3.4 Rhizome and storage roots formation

Underground parts comprised of round rhizome bearing storage roots and fibrous roots. From September to October, the underground parts of the 1st PS was swollen and modified to become the new rhizome as well as the contractile roots were also modified into the new storage roots (Fig 3.6e, Fig 3.8a1-4). Afterwhich the 2nd and 3rd new rhizomes were modified in November (Fig 3.6f).

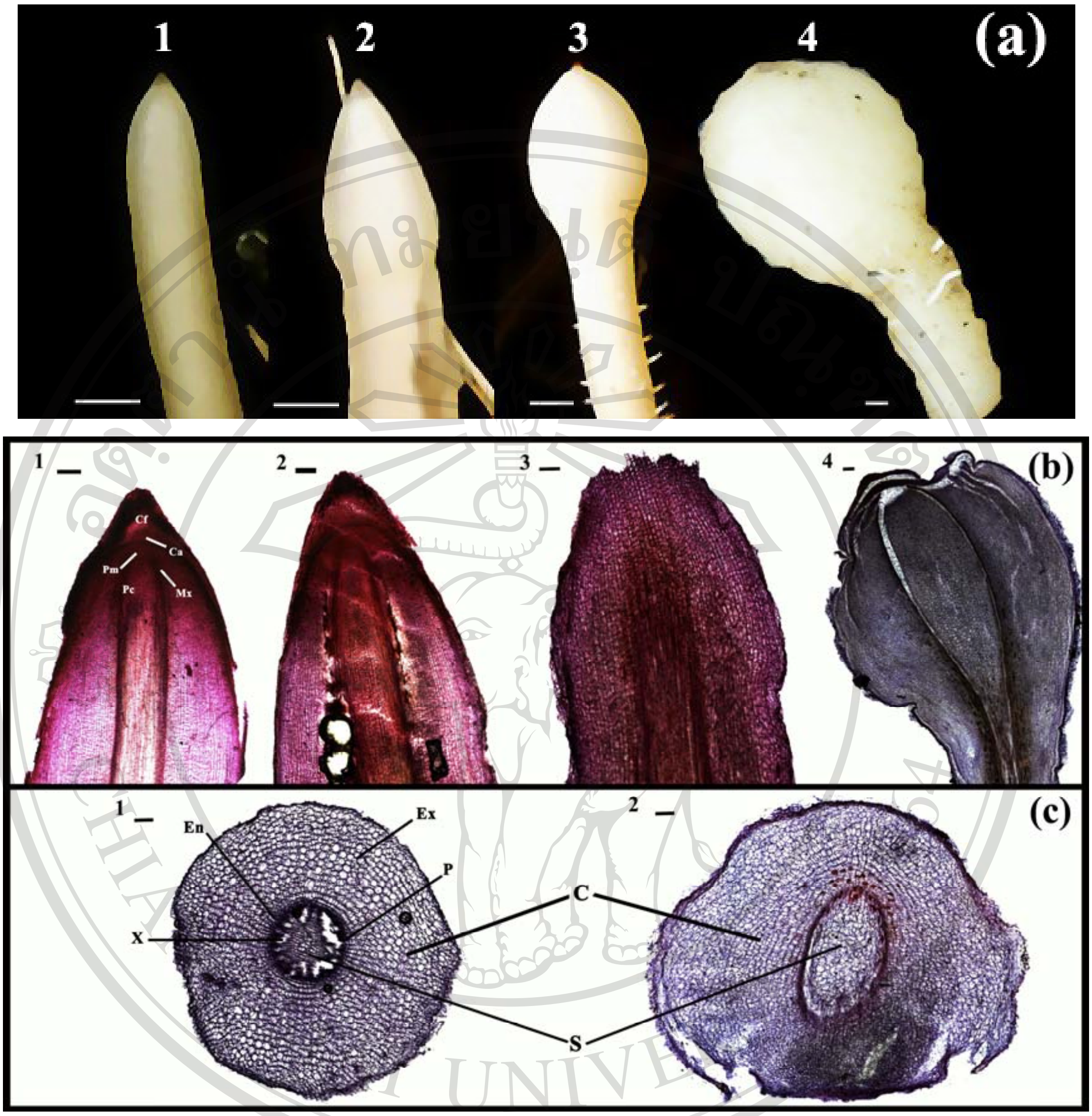

Figure 3.8 Photographs a1-4 were the development of a storage root. Photographs b and c, Longitudinal section (b1-4) and cross section of the storage roots (c1-2) (Cf = root cap; Ca = calyptrogen; Pm = promeristem; Mx = metaxylem; Pc = procambium; En = endodermis; Ex = exodermis; X = xylem; P = pericycle; C = cortex, and S = stele).
Bar = 200 µm.

The diameter of new rhizome was the same as the diameter of pseudo-stem during 1 and 14 WAP and the size was enlarged more rapidly after 14 WAP and reached a maximum at 23 WAP (Fig 3.9a). Contractile roots initiated at 5 WAP with 0.15 – 0.18 cm in diameter. The terminal part of the contractile root was swollen after 11 WAP and gradually modified to become storage roots with 0.47 to 1.6 cm of diameter from 14 to 23 WAP (Fig 3.9b, Fig 3.8a1-4). Cell modification in storage roots was investigated by micro-technique method (Fig 3.8b, 3.8c and 3.10). The results showed that the swelling of storage roots caused by cell division and cell enlargement in cortex and stele (Fig 3.8c), cell width changed rapidly from 14 WAP (78.10 µm) and reached a maximum at 23 WAP (143.51 µm) (Fig 3.9c and Fig 3.10), consistence with the increase of diameter (Fig 3.9b).

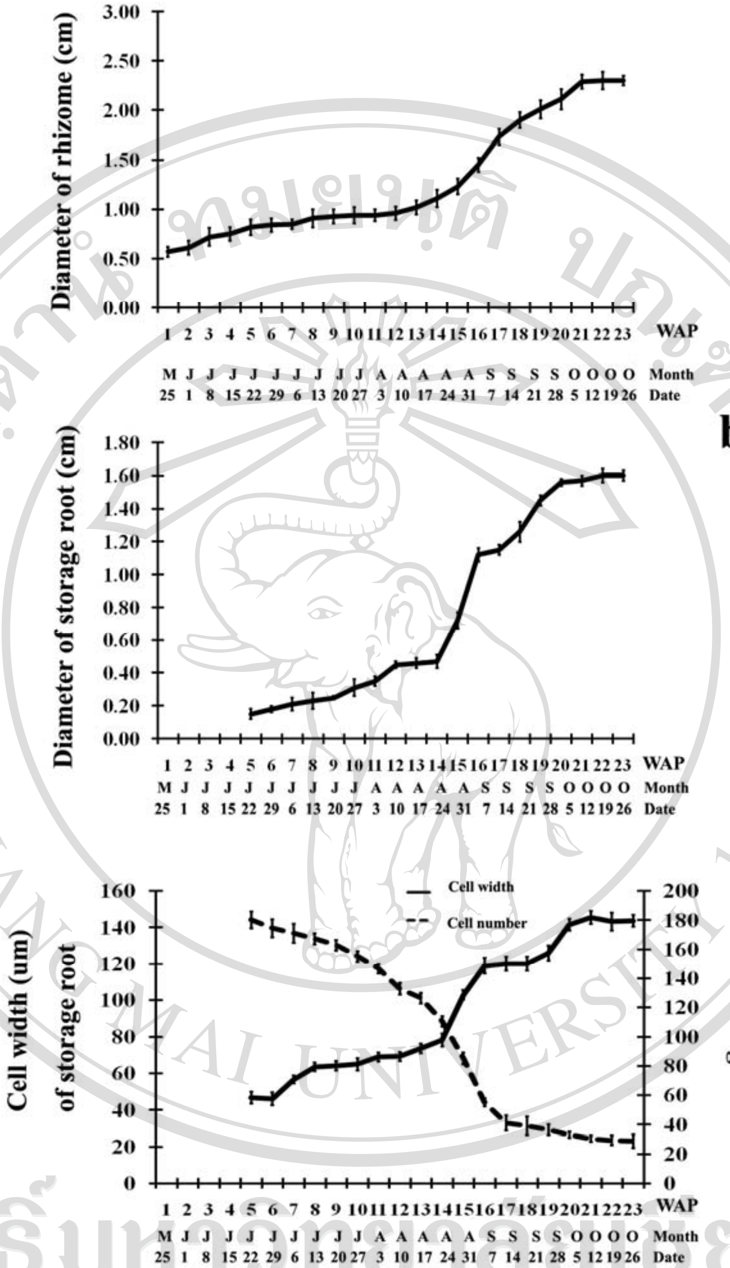

Figure 3.9 Quantification of changes in rhizome and storage root diameter, cell number and cell width during growth and development of *C. alismatifolia* in natural condition.

(a: Diameter of rhizome, b: Diameter of storage roots, c: Cell width and cell number along the transversal axis of the contractile root and storage root, Data were means with standard deviations of five independent measurements.)

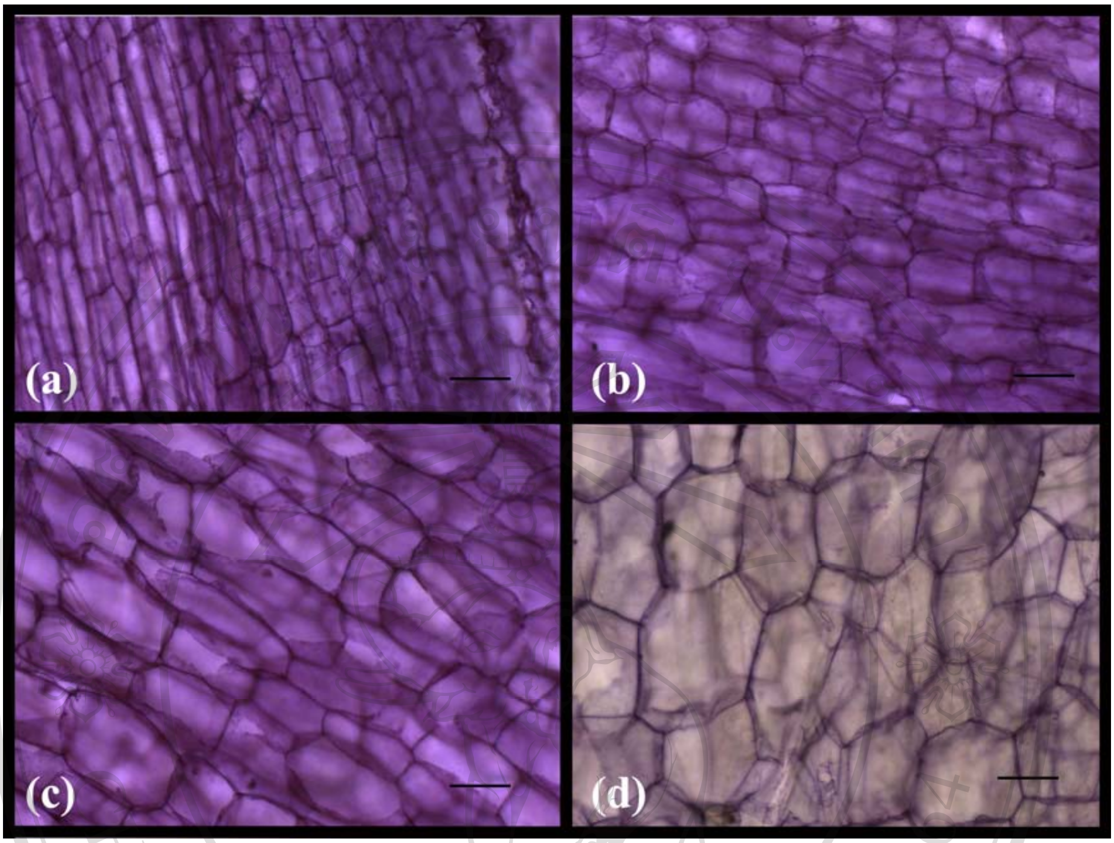

Figure 3.10 Enlargement of parenchymatous cells in cortex of storage roots of *C. alismatifolia* grown under natural conditions. (a = 5 WAP, b = 13 WAP, c = 20 WAP and d = 23 WAP.) Bar = 50 μm

3.3.5 Carbohydrate concentration

Concentration of total nonstructural carbohydrates (TNC) in leaf (mg/gDW) increased from 2 WAP and reached a maximum at 11 WAP (65.32 mg/gDW) and began to decrease from 11 WAP (Fig 3.11a). TNC in rhizome and storage roots rapidly increased from 14 WAP and reached a maximum from 18 to 19 WAP (279.12, 286.51 mg/gDW, respectively) and did not change thereafter (Fig 3.11b and 3.11c).

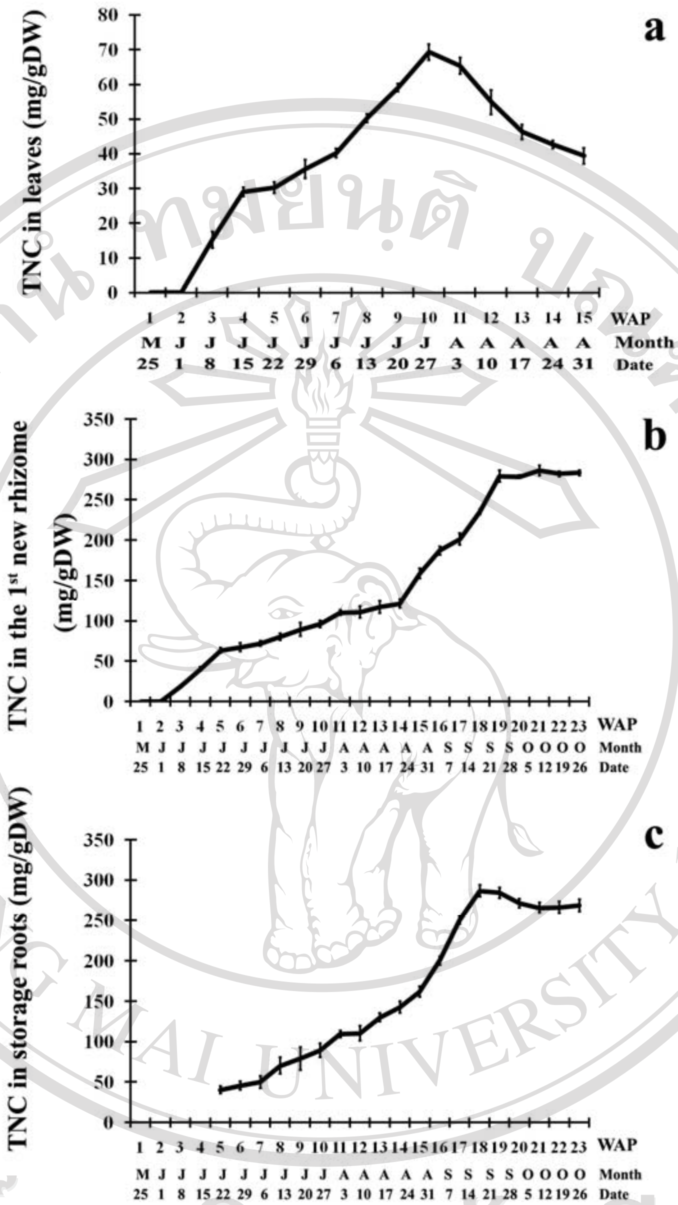

Figure 3.11 Changes of TNC in leaves, rhizome and storage roots during growth and development of natural conditions.
(a: TNC of leaves, b: TNC of rhizome, c: TNC of storage roots Data were means with standard deviations of five independent measurements.)

3.4 Discussion

Under natural conditions in Thailand, the growth cycle of *C. alismatifolia* 'Chiang Mai Pink' started from May to November. The vegetative growth of mother stem terminated at 11 WAP (Aug 3). However, the 2nd and 3rd PS were continuously growing, and flowering occurred in August under 65% RH, 28°C and 12.5 hrs of sunshine duration (Fig 3.2). After this month, sunshine duration gradually shortened and relative humidity decreased until April. Average temperature gradually decreased until December (Fig. 3.2). These conditions may be the important signals to induce mechanisms in plant to survive by developing rhizome formation. Rhizome formation process of *C. alismatifolia* could be derided into 3 stages, i.e. 1) initiation stage, 2) differentiation and development stage, and 3) maturation stage. Initiation of the 1st order rhizome and storage roots formation might start during 11-13 WAP (Aug 3-17) because vegetative growth started to decrease (Fig 3.3) whereas leaves, dry matter and photosynthetic reserve (TNC) in leaves decreased, indicated that translocation of food reserve from sources (leaves) to sink (rhizome and storage roots) started during this period (Fig. 3.4a and 3.11a) and then cell width of storage roots started to increase (Fig. 3.9c). At this period, above ground parts reached it maximum height of 42.40 cm with 3-4 foliage leaves. Therefore, initiation might occur at about 11 WAP when assimilates were translocated to rhizome and storage roots. Differentiation and development of storage organs occurred from cell division and expansion during 14 - 22 WAP (Aug, 24 – Oct, 19). Changes of cell shape and size occurred in this period (Fig. 3.10). Loss of total nonstructural carbohydrate from leaves (aerial parts) occurred at 11 WAP (Fig. 3.11d), and translocated for accumulation in new rhizome and storage roots (underground parts). Ripening stage started at 23 WAP (Oct, 26), when the growth of new rhizome and storage roots were there the maximum, while concentration of food reserve did not change. Similar pattern later occurred in the 2nd and 3rd new rhizome and they became ripen later than the 1st new rhizome. After ripening, underground parts went to rest under cool weather, low relative humidity and short day in November (Fig 3.2). In potatoes, premature tuber formation was correlated with conditions (short day light and low temperature) which checked root and sprout formation after planting, but not necessarily with the loss of reserve materials in the mother tuber (Van Schrseven, 1956). During the period of *C. species* ensuing cold season, starch accumulation starts until November (or up to complete maturity), increasing the weight of the rhizome (Mathai, 1978). Delayed growing of *C. alismatifolia* in October needed the night break treatment to promote flowering and maintain flower qualities. However, off-season production reduced rhizome quality of this plant (Ruamrungsri *et al*., 2006).

Rhizomes and storage roots formation of *C. alismatifolia* consisted of two different cell morphogenetic steps: cell division and cell enlargement. Formal literature, the author describe that cell division precedes cell enlargement (Artschwager, 1924). Figure 3.10a showed that cell division of storage roots was first seen at 5 WAP. After that cell divided transversally, and then elongated. However, these processes were only involved in storage roots elongation. When storage roots started to swell, cell number did not increase. The cell enlargement of storage roots was increased at 13 WAP.

3.5 Conclusion

Growth cycle of *C. alismatifolia* took place from August to November (23 WAP). Process of rhizome formation could be separated into 3 stages, i.e. 1) Initiation stage, 2) differentiation and development, and 3) maturation stage. Initiation stage occurred at 11-13 WAP when vegetative growth was maximal. Cell division and enlargement actively increased during 14-22 WAP so called “differentiation and development” stage. Rhizome maturated at 23 WAP when its growth was terminated. Storage roots were 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, rhizome was harvested at 23 WAP.