

TABLE OF CONTENTS

	page
Acknowledgement	iii
Abstract in Thai	iv
Abstract in English	vi
List of Table	xiv
List of Figures	xvii
List of Abbreviations and Symbols	xxii
Chapter 1 Introduction	1
Chapter 2 Review of Literature	4
2.1 Origin and distribution of <i>C. alismatifolia</i> Gagnep.	4
2.2 Economic important values	4
2.3 Morphology	6
2.3.1 Underground parts	6
2.3.2 Aerial parts	8
2.4 Cultivation	14
2.5 Physiology of rhizome and contractile root formation	19
2.5.1 Environmental factors affecting in rhizome formation	20
2.5.2 Effect of day length or photoperiod	21
2.5.3 Effect of light quality	22
2.5.4 Effect of temperature and humidity	22
2.5.5 Effect of plant growth regulator	23
2.6 Rhizome formation <i>in vitro</i>	23
Chapter 3 Rhizome formation under natural conditions	25
3.1 Introduction	25
3.2 Material and methods	25
3.2.1 Plant materials preparation	25

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

3.2.2	Data collection	26
3.2.2.1	Measurement of vegetative growth	26
3.2.2.2	Growth of cell and tissue development of rhizomes and storage roots	26
3.2.2.3	Analysis of carbohydrate concentration in rhizomes and storage roots	27
3.2.2.4	Meteorological data collection	27
3.3	Results and Discussion	27
3.3.1	Growth of aerial parts (pseudo-stem and flowers)	27
3.3.2	Changes of dry weight	28
3.3.3	Pattern of growth	30
3.3.4	Rhizome and storage roots formation	32
3.3.5	Carbohydrate concentration	36
3.4	Discussion	38
3.5	Conclusion	39
Chapter 4	Rhizome formation <i>in vitro</i>	40
4.1	Introduction	40
4.2	Material and methods	41
4.2.1	Plant material	41
4.2.2	Effect of sucrose concentration	41
4.2.3	Culture conditions	41
4.2.4	Measurements and Statistical Analysis of data	42
4.3	Results	42
4.3.1	Growth of rhizome	42
4.3.2	Contractile root formation	45
4.4	Discussion	46
4.5	Conclusion	48
Chapter 5	Effect of day length on growth and rhizome formation of <i>Curcuma alismatifolia</i>	49
5.1	Introduction	49

5.2 Material and methods	50
5.2.1 Plant materials	50
5.2.2 Data collection	51
5.2.2.1 Recording of vegetative growth	51
5.2.2.2 Photosynthetic efficiency	52
5.2.2.3 Chlorophyll fluorescence	53
5.2.2.4 Measurement of total chlorophyll content	53
5.2.3 Plant analysis	55
5.2.4 Gene expression during the rhizome formation by differential display (DD RT-PCR)	55
5.2.5 Statistical analysis	55
5.3 Results	56
5.3.1 Vegetative growth	56
5.3.2 Development of rhizome and storage roots	61
5.3.3 Photosynthetic efficiency	65
5.3.4 Chlorophyll fluorescence	65
5.3.5 Total chlorophyll concentration	67
5.3.6 Biochemical content in rhizome and storage roots	67
5.3.6.1 Total non structural carbohydrate (TNC) and Starch	67
5.3.6.2 Total soluble sugar (TSS) and reducing sugar (RS)	70
5.3.6.3 Total amino acid concentration	70
5.3.6.4 Other free sugar (Fructose, Glucose and Sucrose)	71
5.3.6.5 Nutrient concentration	72
5.3.7 Gene expression in rhizome under different day length	73
5.4 Discussion	78
5.5 Conclusion	80

Chapter 6	Effect of red light on rhizome formation of <i>Curcuma alismatifolia</i>	82
6.1	Introduction	82
6.2	Material and methods	83
6.2.1	Plant materials	83
6.2.2	Data collection	85
6.2.2.1	Change of vegetative growth and physiological aspects	85
6.2.2.2	Gene expression in the rhizome formation by DD RT-PCR	85
6.2.2.3	Changes in biochemical contents in rhizome and storage roots	85
6.3	Results	85
6.3.1	Plant growth and development	85
6.3.2	Photosynthetic rate and Chlorophyll fluorescent	89
6.3.2.1	Photosynthetic rate	89
6.3.2.2	Chlorophyll fluorescence	91
6.3.3	Biochemical content in rhizome and storage root	92
6.3.3.1	Total nonstructural carbohydrate (TNC)	92
6.3.3.2	Starch concentration	94
6.3.3.3	Total soluble sugar (TSS) concentration	94
6.3.3.4	Reducing sugar (RS) concentration	96
6.3.3.5	Free sugar (Fructose, Glucose and Sucrose)	98
6.3.3.6	Total nitrogen concentration	99
6.3.4	Gene expression during rhizome formation by DD RT-PCR	100
6.4	Discussion	102
6.5	Conclusion	105

Chapter 7	General discussion and conclusion	106
	7.1 General discussion	106
	7.1.1 Physiological mechanism of rhizome under natural environment and <i>in vitro</i> conditions	106
	7.1.2 Effect of day length and red light on growth and rhizome formation	108
	7.1.2.1 Day length	108
	7.1.2.2 Red light	110
	7.2 Conclusion	111
	7.3 Suggestion for future experiment	112
References		113
Appendix		121
Curriculum Vitae		138

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

LIST OF TABLES

Table		page
2.1	The number and economic values of exported <i>C. alismatifolia</i> rhizome from 1997 to 2004.	5
2.2	The number and economic values of exported <i>C. alismatifolia</i> rhizome, ranking by countries.	5
2.3	The number and economic values of exported <i>C. alismatifolia</i> inflorescences.	6
2.4	The number and economic values of exported <i>C. alismatifolia</i> inflorescences, ranking by countries in 2004.	6
4.1	Effect of sucrose concentrations on <i>in vitro</i> shoot and rhizome inductions of <i>C. alismatifolia</i> Gagnep cultured on MS medium (induction period: 8 months).	45
4.2	Contractile root of <i>C. alismatifolia</i> Gagnep cultured on MS medium different sucrose concentrations (induction period: 8 months).	46
5.1	Effect of day lengths (7, 10, 13 hrs and control) on plant height, diameter of pseudo-stem, number of leaves per plant cultured at 15 WAP.	59
5.2	Effect of day length on number and quality of rhizomes and storage roots at harvest.	64
5.3	Characteristics of chlorophyll in plants grown under different light sources at 5 WAP.	67
5.4	Total non-structural carbohydrates (TNC), starch and total nitrogen in new rhizomes and storage roots of <i>C. alismatifolia</i> under different day length treatments at harvest.	68

5.5	Reducing sugars (RS) and total soluble sugars (TSS) in new rhizomes and storage roots of <i>C. alismatifolia</i> under different day length treatments at harvest.	70
5.6	Total amino acids in new rhizomes and storage roots of <i>C. alismatifolia</i> under different day length treatments at harvest.	71
5.7	Free sugars (Fructose, Glucose and Sucrose) in new rhizomes and storage roots of <i>C. alismatifolia</i> grown under different day light treatments at harvest.	72
5.8	Concentrations of nutrients in new rhizomes and storage roots under different day length treatments at harvest.	73
5.9	The nucleotide sequences isolated from rhizome of <i>C. alismatifolia</i> grown under 13 hrs day length.	76
5.10	List of differentially expressed fragments isolated from <i>C. alismatifolia</i> in comparison to other plant genes.	77
6.1	Growth of <i>C. alismatifolia</i> grown under different light sources at 11 WAP.	87
6.2	Effect of different light sources on yield and qualities of new rhizomes and storage roots of <i>C. alismatifolia</i> at harvest.	89
6.3	Photosynthetic rates of plants grown under different light sources at 5 WAP.	91
6.4	Characteristics of chlorophyll in plants grown under different light sources at 5 WAP.	92
6.5	Total non-structural carbohydrates (TNC) and starch in new rhizomes and storage roots of <i>C. alismatifolia</i> under different light source treatments at harvest.	96
6.6	Reducing sugars (RS) and total soluble sugars (TSS) in new rhizomes and storage roots of <i>C. alismatifolia</i> under different light source treatments at harvest.	98

- 6.7 Total nitrogen in new rhizomes and storage roots of *C. alismatifolia* under different light source treatments at harvest. 100



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF FIGURES

Figure		page
2.1	Fibrous roots (a) and contractile roots (b) of <i>C. alismatifolia</i> .	7
2.2	Rhizome (a) and storage roots (b) of <i>C. alismatifolia</i> .	8
2.3	Pseudo-stem (a) and foliage leaves (b) of <i>C. alismatifolia</i> .	9
2.4	Compact spike (a) and pink coma bracts in upper part (b) of plant.	10
2.5	True flower of <i>C. alismatifolia</i> .	11
2.6	Fruit (a, b) and seed (c) of <i>C. alismatifolia</i> . (Kirkbride <i>et al.</i> , 2007)	13
3.1	The rhizomes (a) and plantlets (b) of <i>C. alismatifolia</i> growing at Lampang province, Thailand.	26
3.2	Average monthly temperature, relative humidity and sunshine duration in 2004 at Lampang province, Thailand.	27
3.3	Characteristics of plants grown under natural environmental condition. a: plant height, b: number of leaves per plant, c: diameter of pseudo-stem, d: number of new shoots per cluster. Data are means with standard deviations of ten independent measurements	28
3.4	Dry weight of leaves (a), 1 st rhizome (b) and storage roots (c) grown under natural environmental conditions. Data were means with standard deviations of ten independent measurements.	29
3.5	The 2 nd lateral pseudo-stem emerged at 6 WAP (a) and 7 WAP (b).	30

- 3.6 Flow diagram illustrating the development of *C. alismatifolia*. 31
 ORh = old rhizome, PS = pseudo-stem, F = flowering, CR = contractile root, NRh = new rhizome and SR = storage root. Size of the circles indicates the relative size of the plant compartment. Arrows indicate the direction of allocation. Thickness of the arrows indicates the relative strength of the allocation.
- 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)). 32
 Bar = 200 μ m
- 3.8 Photographs a1-4 were developed of storage root. Photographs b and c, Longitudinal section (b1-4) and cross section of storage roots (c1-2) (Cf = root cap; Ca = calyptragen; Pm = promeristem; Mx = metaxylem; Pc = procambium; En = endodermis; Ex = exodermis; X = xylem; P = pericycle; C = cortex, and S = stele). Bar = 200 μ m. 33
- 3.9 Quantification of changes in rhizome and storage root diameter, cell number and cell width during natural condition. 35
 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.
- 3.10 Enlargement of parenchyma cells in cortex of storage roots 36
 grown under natural conditions.
 (a = 5 WAP, b = 13 WAP, c = 20 WAP and d = 23 WAP.)
 Bar = 50 μ m

3.11	Changes of TNC in leaves, rhizomes and storage roots diameter, cell number and cell width during natural conditions. a: TNC of leaves, b: TNC of rhizome, c: TNC of storage roots Data are means with standard deviations of five independent measurements.	37
4.1	The young shoots of <i>C. alismatifolia</i> were cultured on MS medium with free plant growth regulator.	42
4.2	Culture of <i>C. alismatifolia</i> Gagnep on <i>In vitro</i> . (a) Shoot multiplication on MS medium containing 6, 5, 4 and 3% sucrose concentration (induction period: 6 months). (b) Browning of plantlet grew on MS medium containing 6% sucrose concentration (growing period: 6 months). (c) New rhizome and contractile roots cultured on MS medium containing different sucrose concentrations [3, 4, 5 and 6%]	44
4.3	Contractile roots culture of <i>C. alismatifolia</i> Gagnep on MS medium containing 4% sucrose [(a) at 6 months and (b) 8 months].	46
5.1	Plant materials (a) grown in a growth chamber (b).	52
5.2	Measurement of chlorophyll fluorescence.	53
5.3	Measurement of chlorophyll content using SPAD	54
5.4	Plants height, number of leaves per plant and diameter of pseudo-stem under different day length conditions (7, 10, 13 hrs and control).	57
5.5	Plants under different day length conditions (a: at 7, 10 and 13 hr) compared with natural environmental condition (b) at 4 WAP.	58
5.6	Plant height and pseudo-stem sizes under different day length conditions (a: at 7, 10 and 13 hrs) compare with natural environmental condition (b) at 12 WAP.	58
5.7	Inflorescence of plants under 7 hrs (a and b) compared with controlled treatment (c) at 6 WAP.	60

5.8	Inflorescences under 13 hrs of day length (a) at 8 WAP compared with controlled treatment (b) at 10 WAP.	61
5.9	Development of rhizomes and storage roots under 10 hrs day length condition at 5 WAP (a) and 7 WAP (b).	62
5.10	Rhizomes and storage roots grown under 7 (a), 10 (b), 13 hr (c) day length conditions compared with controlled treatment (d) at harvest.	63
5.11	Photosynthetic rate (a) and Chlorophyll fluorescence (b) of plant growing under different day lengths compared with controlled treatment. Error bars denote the SE(n=10). * indicated significant differences at $P < 0.05$.	66
5.12	Morphology of starch in rhizomes and storage roots of <i>C. alismatifolia</i> under controlled treatment at harvest.	69
5.13	The total RNA of 7 and 13 hrs day lengths as extracted by Phenol/SDS method (a) and cleaned up by RNeasy [®] Kit (b). Agarose gel electrophoresis analysis of the nested PCR products rhizome of curcuma grown under different light sources (7 and 13 hrs) (c).	75
6.1	Plant materials (a) grown under red light (b) cool day light (c) and natural light (d).	84
6.2	Growth characteristics of plants grown in different light sources.	86
6.3	Flowering of plants grown under red light [(a) right] and cool day light [(a) left] conditions at 4 WAP. Plant grown under controlled treatment did not flower at 4 WAP (b). Flowers of plants grown under red light at 4 WAP (c). [bar = 1 cm]	88

6.4	Photosynthetic rates of <i>C. alismatifolia</i> grown in different light sources.	90
6.5	Chlorophyll fluorescence of <i>C. alismatifolia</i> grown under different light sources.	91
6.6	The total non-structural carbohydrates (TNC) of rhizomes (a) and storage roots (b) grown under different light sources.	93
6.7	Carbohydrate content in the new rhizome: a) and storage roots ; b) of <i>C. alismatifolia</i> on different light sources.	95
6.8	The reducing sugars of rhizomes and storage roots grown under different light sources.	97
6.9	Fructose glucose and sucrose contents in the new rhizome (a) and storage roots (b) of <i>C. alismatifolia</i> on different light source.	99
6.10	The total RNA of red light and natural light as extracted by Phenol/SDS method (a) and cleaned up by RNeasy [®] Kit (b). Agarose gel electrophoresis analysis of the nested PCR products rhizome of curcuma grown under different light sources (Red light; R and Natural light; N)(c).	101

List of Abbreviations and Symbols

BA	: 6-benzylaminopurine	mg	: Milligram
°C	: Degree Celsius	ml	: Milliliter
cm	: Centimeter	MS	: Murashige and Skoog
DW	: Dry weight	PAR	: Photosynthetically active radiation
FAA	: Formalin-acetic acid alcohol	<i>Pn</i>	: Photosynthetic rate
<i>F_m</i>	: Maximal fluorescence	RH	: Relative humidity
<i>F_o</i>	: Minimal fluorescence	rpm	: Round per minute
<i>F_v</i>	: Variable fluorescence	RS	: Reducing sugar
<i>F_m/F_v</i>	: Optimum quantum yield of PS II	TNC	: Total non-structural carbohydrate
g	: Gram	TSS	: Total soluble sugar
hrs	: Hours	WAP	: Week after planting
l	: Liter	%	: Percent

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved