IDENTIFICATION OF CHEMICALS AND CATALOGING OF GENES INVOLVED IN THE HYPOCOTYL ELONGATION OF *Arabidopsis* UNDER CONTINUOUS BLUE LIGHT

ONG WEN DEE

UNIVERSITI SAINS MALAYSIA

2017

IDENTIFICATION OF CHEMICALS AND CATALOGING OF GENES INVOLVED IN THE HYPOCOTYL ELONGATION OF *Arabidopsis* UNDER CONTINUOUS BLUE LIGHT

by

ONG WEN DEE

Thesis submitted in fulfillment of the requirements

for the degree of

Doctor of Philosophy

August 2017

ACKNOWLEDGEMENT

First of all I thank God for His love and encouragement. I am also very thankful to many people who have supported and encouraged me towards the completion of this thesis.

I sincerely thank my supervisor, Prof. Sudesh Kumar for accepting me as a student and providing me the opportunity to complete my graduate work with you and your collaborators in Japan. Thank you for your confidence in my abilities and my deepest acknowledgement of your unwavering encouragements and advice throughout the progression of this project.

I am very grateful to Prof. Minami Matsui for accepting me to conduct my doctoral research in his laboratory in RIKEN. I am thankful for his support and contribution towards the success of this project. Your opinions and suggestions have contributed to a better experiment.

A special appreciation to fellow researchers including Dr. Yukio Kurihara, Dr. Setsuko Shimada, Dr. Emiko Kurihara and Ms. Mika Kawashima for assisting me with the laboratory work and for spending your time clearing my doubts. I thank you for providing me the assistance to solve any technical difficulties and for the goodwill you showed to me. I also wish to express my gratitude to Dr. Yukio Kurihara and Dr. Yoshito Oka for providing me important comments and assistance in the research publication.

Finally, I wish to express my deepest thanks to my family for giving me full support, love and understanding all this while.

TABLE OF CONTENTS

Acknowledgement **ii**

Table of Contents **iv**

List of Tables **x**

List of Figures **xi**

List of Formulas **xiii**

List of Abbreviations **xiv**

List of Symbols **xx**

List of Units **xxi**

Abstrak **xxiii**

Abstract **xxvi**

CHAPTER 1 INTRODUCTION **1**

1.1 Introduction and Research Objectives 1

CHAPTER 2 LITERATURE REVIEW **8**

2.1 *Arabidopsis thaliana* (*Arabidopsis*) 8

2.2 The Role of Light in the Life Cycle of Plants 12

2.3 Skotomorphogenesis and Photomorphogenesis 13

2.3.1 Etiolation 15

2.4 Plant Photoreceptors 18

2.4.1 Phytochromes (PHYs) 19

2.4.2 Cryptochromes (CRYs) 22

2.4.3 Phototropins (PHOTs) 25

2.4.4 ZEITLUPE (ZTL) Family 28

2.4.5 UV RESISTANCE LOCUS 8 (UVR8) 31

2.5 Functions of Photoreceptors in *Arabidopsis* 34

2.5.1 Seed Germination 35

2.5.2 Shade Avoidance 37

2.5.3 Phototropism 38

2.5.4 Chloroplast Movement and Stomatal Opening 40

2.5.5 Flowering Time 42

2.5.6 Circadian Clock 43

2.6 Photomorphogenesis Controlled by Photoreceptors 46

2.7 Chemicals and Applications in Plant Research 49

CHAPTER 3 CLASSIFICATION OF CHEMICALS BASED ON MORPHOLOGY OF SEEDLINGS 55

3.1 Introduction 55

3.1.1 Forward and Reverse Chemical Screening 55

3.1.2 *Arabidopsis* Early Developmental Switches: From Dry Seed to Photoautotrophic Seedling 60

3.1.3 High-content Chemical Screening 65

3.2 Materials and Methods 67

3.2.1 Medium, Plant Material and Sample Preparation 67

3.2.2 Chemicals 68

3.2.3 Light Chamber and Plant Growth Condition 69

3.2.4 Liquid Assay Screening 70

3.2.5 Primary Liquid Assay Screening 70

3.2.6 Liquid Assay Screening for Specific Light Inhibitors 72
3.2.7 Liquid Assay Screening for Light-Dependent Growth Inhibitors 72
3.2.8 Image Acquisition and Extraction of Morphological Data 74
3.2.9 Meta-Analysis of Morphological Data 74
3.2.10 Measurement of Hypocotyl Length for Liquid Assay 75
3.3 Results 76
3.3.1 Primary Liquid Assay Screening 76
3.3.2 LATCA Chemicals Characterization Based on Seedlings Morphology 79
3.3.3 Hypocotyl Length Difference between Internal Control and Controls 81
3.3.4 Inhibitors of Specific Light Signal 82
3.3.5 Inhibitors of Seedlings Growth 84
3.3.6 The Effect of Chemical Dosage and Light Type to Seedlings Growth 85
3.4 Discussion 90
3.5 Conclusion 100

CHAPTER 4 CHARACTERIZATION OF LOSS-OF-FUNCTION MUTANTS ON 3B7N REGULATION OF HYPOCOTYL ELONGATION 102

4.1 Introduction 102
4.1.1 Revealing Gene Function through Mutation 102
4.1.2 Cryptochromes and Its Mechanism 103
4.1.3 Main Interacting Components of Photomorphogenesis 105

4.2 Materials and Methods 109

4.2.1 Agar Medium, Plant Materials and Sample Preparation 109

4.2.2 Chemicals and Analogs 110

4.2.3 Light Chamber and Growth Condition 110

4.2.4 Screening *Arabidopsis* WT with 3B7N under Various Lights 111

4.2.5 Screening of 3B7N Concentration and Blue Light Intensity Effect on *Arabidopsis* WT 111

4.2.6 Screening *Arabidopsis* WT with 3B7N and Analogs 112

4.2.7 Screening *Arabidopsis* WT with Plant Nitric Oxide (NO) Inhibitors 112

4.2.8 Screening Mutants and Over-expressors with 3B7N 113

4.2.9 Image Acquisition 113

4.2.10 Measurement of Hypocotyl Length 113

4.2.11 Measurement of Chemical Inhibition of Light Signal 114

4.2.12 Statistical Analysis 114

4.3 Results 116

4.3.1 The Inhibition of 3B7N on Blue Light-mediated Suppression of Hypocotyl Elongation 116

4.3.2 Effect of 3B7N Concentration and Blue Light Intensity on Hypocotyl Elongation 118

4.3.3 The Effect of Plant Nitric Oxide (NO) Inhibitors on Hypocotyl Length 120

4.3.4 The Effect of 3B7N and Its Analogs on Hypocotyl Length 122

4.3.5 The Effect of 3B7N and Analogs on Inhibition of Blue Light Signal 124

4.3.6 The Effects of 3B7N Analog Structures on Hypocotyl Elongation 124

4.3.7 Hypocotyl Length Responses of Photoreceptors Mutants and Over-expressors to 3B7N 127

4.3.8 Hypocotyl Length Responses of Blue-light Signaling Components Mutants to 3B7N 131

4.3.9 The Effect of 3B7N on Cotyledon in Blue Light 134

4.4 Discussion 136

4.5 Conclusion 144

CHAPTER 5 PHYSIOLOGY ASSESSMENT OF 3B7N IN THE REGULATION OF HYPOCOTYL ELONGATION USING MICROARRAY ANALYSIS 145

5.1 Introduction 145

5.1.1 Regulation of Gene Expression by Light 145

5.1.2 Transcriptomic for Dissection of Chemical Function 148

5.2 Materials and Methods 150

5.2.1 Chemicals, Plant Materials and Growth Conditions 150

5.2.2 Experimental Design 151

5.2.3 RNA Extraction 151

5.2.4 RNA Purification 152

5.2.5 cRNA Construction 152

5.2.6 Labeled cRNA Purification and Quantification 154

5.2.7 cRNA Fragmentation 155

5.2.8 Microarray Hybridization 155

5.2.9 Microarray Chip Scanning and Signal Acquisition 156

5.2.10 Trimming and Analysis of Microarray Data 156

5.2.11 Gene Ontology (GO) Annotation and Protein Classification 157

5.3 Results 159

5.3.1 Expression Profiles of *Arabidopsis* Genes Induced by Blue-light Chemical Inhibitors 159

5.3.2 Expression Profiles of *Arabidopsis* Genes by 3B7N in *cry1cry2* Mutant 168

5.3.3 Expression Patterns of Blue Light-Specific Genes Treat with 3B7N 170

5.3.4 Gene Ontology Annotation and Protein Classification of the Genes Significantly Induced by 3B7N 172

5.4 Discussion 176

5.5 Conclusion 186

CHAPTER 6- GENERAL DISCUSSION 184

CHAPTER 7 - CONCLUSION 194

REFERENCES 197

APPENDICES

LIST OF PUBLICATIONS

LIST OF TABLES

		Page
Table 3.1	Chemicals from the primary screening with inhibition effect on *Arabidopsis* seed germination or seedling growth	77
Table 3.2	The hypocotyl length of the internal control, mean length of controls and the percentage change of the hypocotyl in various light irradiations	82
Table 3.3	Chemicals showing significant effect on hypocotyl length under specific light irradiation	84
Table 4.1	List of chemicals used for hypocotyl evaluation	111
Table 4.2	The hypocotyl length, hypocotyl growth percentage and the blue light inhibition strength of chemicals with elongated hypocotyl	124
Table 5.1	The components and volume for cRNA construction	153
Table 5.2	The buffer and volume used for cRNA hybridization.	155
Table 5.3	List of *Arabidopsis* genes that were induced by 3B7N in blue light with expression fold more or equal to 5-fold	162
Table 5.4	List of *Arabidopsis* genes induced by A2 with expression change more than 2-fold compared to DMSO	168
Table 5.5	Regulation status of known blue-light genes and transcription factors in presence of 3B7N	171

LIST OF FIGURES

		Page
Figure 2.1	Schematic timelime growth of the chronological progression of principal *Arabidopsis* growth stages	9
Figure 2.2	Comparison of *Arabidopsis* seedlings grown in dark and in the presence of light	14
Figure 2.3	The domain and structure of *Arabidopsis* PHYs	20
Figure 2.4	Domain and structure of *Arabidopsis* CRYs	24
Figure 2.5	*Arabidopsis* PHOTs	26
Figure 2.6	Domain and structure of the ZTL family photoreceptors	29
Figure 2.7	UVR8 homodimer and the monomer and structure-function	32
Figure 2.8	Molecular interaction of UVR8 in response to UV-B light	33
Figure 2.9	Light-dependent plant development mediated by photoreceptors in *Arabidopsis*	36
Figure 2.10	Hypocotyl phototropic curvature responses of phototropin mutants of *Arabidopsis*	39
Figure 2.11	Flowering time response of *Arabidopsis* WT and photoreceptor mutants	44
Figure 2.12	Five-day-old seedlings grown in various light conditions	47
Figure 3.1	Comparison of forward and reverse chemical screening	56
Figure 3.2	Comparison of morphological and physiological key processes during seed germination	62
Figure 3.3	Illustration of the liquid assay chemical screening on *Arabidopsis*	71
Figure 3.4	The 96-well format for liquid assay screening	73
Figure 3.5	The scatter plot representing the effect of LATCA chemicals on seedling growth	80
Figure 3.6	Seedling images with growth inhibitors at 5 μM in monochromatic blue or red lights	86

Figure 3.7	*Arabidopsis* seedlings images with treatment growth inhibitors at different concentrations	87
Figure 3.8	Seedling images with growth inhibitors of lower concentrations	89
Figure 4.1	Signal transduction mechanism of *Arabidopsis* CRY	104
Figure 4.2	Hypocotyl length responses of WT Col-0 seedlings at 2.5 μM 3B7N in various monochromatic lights.	117
Figure 4.3	The profile of hypocotyl lengths of WT Col-0 seedlings in different blue light intensity and 3B7N concentration.	119
Figure 4.4	Hypocotyl lengths of WT Col-0 seedlings treated with 3B7N and plant nitric oxide inhibitors at 5 μM in blue light	121
Figure 4.5	Hypocotyl lengths of WT Col-0 seedlings treated with 3B7N and its analogs.	123
Figure 4.6	Chemical structures of 3B7N and its analogs and the hypocotyl elongation responses of WT Col-0 *Arabidopsis* seedlings in blue light	126
Figure 4.7	Hypocotyl length responses of mutants and over-expressors by treatment of 3B7N in continuous blue light	128
Figure 4.8	Hypocotyl elongation responses of cryptochrome mutants and over-expressors in 3B7N treatment under different light conditions	130
Figure 4.9	Hypocotyl elongation responses of COP1 and HY5 mutants in 3B7N treatment under continuous blue light	132
Figure 4.10	Hypocotyl elongation responses of known light signaling transcription factors mutantsin 3B7N treatment under continuous blue light	133
Figure 4.11	Cotyledon size of WT Col-0 6-day-old seedlings with 3B7N	135
Figure 5.1	Gene expression profile changes of *Arabidopsis* WT Col-0 after treatment with 3B7N and derivatives.	160
Figure 5.2	Microarray analysis of *Arabidopsis* WT and *cry1cry2* seedlings with 3B7N treatment under bluelight irradiation	169
Figure 5.3	GO classification of genes significantly induced by 3B7N in WT under continuous blue light	173
Figure 5.4	Protein classification of *Arabidopsis* genes significantly induced by 3B7N	175

LIST OF FORMULAS

		Page
Formula 1	Z-score = (X-N) / s	75
Formula 2	T-score = 10 (Z-score) + 50	75
Formula 3	Hypocotyl length change (%) = 100 (x-y) / y	75
Formula 4	Inhibition level = (x-y) / (z-y)	114
Formula 5	Yield of purified cRNA (µg) = [(C) x (D)] 1000	155
Formula 6	Specificity activity (pmoL/µg) = [(A) x 1000] / (C)	155

LIST OF ABBREVIATIONS

A1	3-bromoindazole
A2	7-nitroindazole
A3	1-[2-(trifluoromethyl)phenyl]imidazole
A4	3-bromo-4-nitro-1H-indazole
A5	3-bromo-1H-indazole-7-carbonitrile
A6	3-iodo-7-nitroindazole
A7	3-bromo-6-nitroimidazo[1,2-a]pyridine
ABA	abscisic acid
Arg	arginin
AS	asparagine synthetase
ATP	adenosine triphosphate
bHLH	basic helix loop helix
BIC	blue-light inhibitor of *cryptochromes*
BITI	BLUE INSENSITIVE TRAITS 1
BR	brassinosteroid
bZIP	basic leucine zipper
CAB	chlorophyll-a/b-binding protein
$CaCl_2 \cdot 2H_20$	calcium chloride dihydrate
CAT	catalase
CCA1	CIRCADIAN CLOCK ASSOCIATED 1
CCT	carboxyl terminal extension
CDF	CYCLING DOF FACTOR
cDNA	complementary deoxyribonucleic acid

CHS	chalcone synthase
CIB	CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX
CML	calmodulin-like
CO	CONSTANS
$CoCl_2 \cdot 6H_2O$	cobalt (II) chloride hexahydrate
Col	Columbia
COP	CONSTITUTIVE PHOTOMORPHOGENIC
CPD	cyclobutane pyrimidine dimer
C-PTIO	2-(4-carboxyphenyl)- 4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide
cRNA	complementary ribonucleic acid
CRY	cryptochrome
CRY-DASH	cryptochrome–*Drosophila*, *Arabidopsis*, *Synechocystis*
C-terminal	carboxyl terminal
$CuSO_4 \cdot 5H_2O$	copper (II) sulfate pentahydrate
DEA-NONOate	diethylamine NONOate sodium
DET	DE-ETIOLATED
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA-Na_2	disodium ethylenediaminetetraacetatedihydrate
EE	EVENING ELEMENTS
EMS	ethyl methanesulfonate
EtOH	ethanol
FAD	flavin adenine dinucleotide

FC	fold change
$FeSO_4 \cdot 7H_2O$	iron (II) sulfate heptahydrate
FKF1	FLAVIN BINDING KELCH REPEAT F-BOX 1
FMN	flavin mononucleotide
FT	Flowering Locus T
FUS	FUSCA
GA	gibberellin
GABI-Kat	Kōlner *Arabidopsis* T-DNA lines
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GBF1	G-BOX BINDING FACTOR 1
H_3BO_3	boric acid
HFR1	LONG HYPOCOTYL IN FAR-RED 1
HIR	high irradiance response
HKRD	histidine kinase–related domain
HNA	2-hydroxy-1-naphthaldehyde
HNC	2-hydroxy-1-naphthoic acid
HY5	LONG HYPOCOTYL 5
HYH	HOMOLOG OF HY5
IAA	indole-3-acetic acid
Int	intron
JA	jasmonate acid
KDR	KIDARI
KH_2PO_4	monopotassium phosphate
KI	potassium iodide
KNO_3	potassium nitrate

LAF1	LONG AFTER FAR-RED LIGHT 1
LATCA	Library of Active Compounds on *Arabidopsis*
LD	long day
LED	light-emitting diode
LFR	low fluence response
LHY	LATE ELONGATED HYPOCOTYL
LKP2	LOV KELCH REPEAT PROTEIN 2
L-NAME	Nω-nitro-l-arginine methyl ester hydrochloride
LOV	Light, Oxygen, or Voltage
MES	2-(N-morpholino)ethanesulfonic acid
$MgSO_4 \cdot 7H_2O$	magnesium sulfate heptahydrate
$MnSO_4 \cdot 4\ H_2O$	manganese (II) sulfate tetrahydrate
mRNA	messenger ribonucleic acid
MTFG	5,10-methenyltetrahydrofolate
MTHF	5,10-methenyltetrahydrofolic acid
MS	Murashige and Skoog
MYBH	MYB-like
NAC	NAM (no apical meristem), ATAF (*Arabidopsis* transcription activation factor), CUC (cup-shaped cotyledon)
$Na_2MoO_4 \cdot 2H_2O$	sodium molybdatedihydrate
NASC	Nottingham *Arabidopsis* Stock Centre
NH_4NO_3	ammonium nitrate,
NO	nitric oxide
NPH3	NON-PHOTOTROPIC HYPOCOTYL 3
N-terminal	amino terminal

PAL	phenylalanine ammonia-lyase
PAS	Period-ARNT-Single-minded
PHL1	PHOSPHATE RESPONSE1-LIKE 1
PIF	PHYTOCHROME INTERACTING FACTOR
PHOT	phototropin
PHR	photolyase homology region
PHY	phytochrome
POR	protochlorophyllide reductase
Pro	promoter
PP2Cs	protein phosphatases 2C
PRR	PSEUDO-RESPONSE REGULATOR
PYL	pyrabactin resistance-like
PYR1	pyrabactin resistance 1
RBCS	ribulose-1,5-bisphosphate carboxylase
RNA	ribonucleic acid
ROP	Rho-of-plants
ROS	reactive oxygen species
RUP	REPRESSOR OF UV-B PHOTOMORPHOGENESIS
SA	salicylic acid
SASSC	SENDAI *Arabidopsis* Seed Stock Center
SAUR	Small auxin up RNA
SD	short day
SDS	sodium dodecyl sulfate
SIR	sirtinol resistant 1
SnRK2	SNF1-related protein kinase 2

SPA	SUPPRESSOR OF PHYTOCHROME A
TAIR	The *Arabidopsis* Information Resource
T-DNA	transferdeoxyribonucleic acid
TOC1	TIMING OF CAB EXPRESSION 1
TRI101	trichothecene 3-O-acetyltransferase
Trp	tryptophan
TUBB1	tubulin beta-1
UV	ultraviolet
UVR8	UV RESISTANCE LOCUS 8
VLFR	very-low-fluence response
v/v	volume over volume
Ws	Wassilewskija
WT	wild type
w/v	weight over volume
$ZnSO_4 \cdot 7H_2O$	zinc sulfate heptahydrate
ZTL	ZEITLUPE
3B7N	3-bromo-7-nitroindazole

LIST OF SYMBOLS

%	percentage
>	more than
≥	equal or more than
<	less than
≤	equal or less than
=	equal to
±	plus or minus
+/-	increase or decrease

LIST OF UNITS

˚C	degree celcious
cm	centimeter
g	gram
h	hour
kDa	kilo dalton
L	liter
M	Molar
Mbp	mega base pair
mg	milligram
min	minute
mL	milliliter
mm	milimeter
nm	nanometer
ng	nanogram
pmoL	picomole
rpm	rotation per minute
sec	second
µg	microgram
µL	microliter
µm	micrometer
µM	micromolar
µmoL	micromole
µmoL $m^{-2}s^{-1}$	micromoles per square meter per second

X time

IDENTIFIKASI BAHAN KIMIA DAN PENGKATALOGAN GEN YANG TERLIBAT DALAM PEMANJANGAN HIPOKOTIL *Arabidopsis* DI BAWAH CAHAYA BIRU YANG BERTERUSAN

ABSTRAK

Tumbuh-tumbuhan mempunyai kemampuan yang luar biasa untuk menerima dan bertindak balas terhadap pelbagai panjang gelombang cahaya dan memulakan pengawalaturan laluan serta komponen molekul isyarat cahaya yang berbeza. Walaupun persepsi cahaya merah dan mekanisme isyarat yang melibatkan fitokrom (PHY) sudah dikenalpasti, pengetahuan terhadap cahaya biru dan mekanisme kriptokrom (CRY) yang mengawal perkembangan anak benih masih tidak difahami sepenuhnya. Kajian ini bertujuan untuk mengenalpasti bahan kimia yang mengawalatur fotomorfogenik yang dikawal oleh CRY dan mengkatalogkan gen yang terlibat dalam pemanjangan hipokotil *Arabidopsis* yang didorong oleh bahan kimia. Penemuan kajian ini menyumbang kepada penemuan yang lebih baik peranan kriptokrom dalam pertumbuhan tumbuhan. Bahan kimia Perpustakaan Bahan Kimia Aktif ke atas *Arabidopsis* (LATCA) telah digunakan dan diperiksa ke atas benih *Arabidopsis* jenis liar (WT) Columbia (Col-0). Bahan kimia yang merangsang pemanjangan hipokotil hanya dalam cahaya biru telah dipilih melalui perbandingan perubahan panjang hipokotil dalam keadaan cahaya yang berbeza. Mekanisme molekul penggalakkan pemanjangan hipokotil yang dikawal oleh cahaya biru dikaji melalui pencirian respon kepanjangan hipokotil mutan hilang fungsi terhadap bahan kimia. Perubahan pada expresi gen *Arabidopsis* yang didorong oleh bahan kimia yang berfungsi khususnya dalam cahaya biru diperiksa mengggunakan mikroarai. Berdasarkan tindak balas kotilidon and hipokotil anak benih *Arabidopsis* jenis liar

yang berumur 6-hari dan dirawat dengan 3,680 bahan kimia, bahan kimia LATCA dibahagikan kepada empat kumpulan: Kumpulan A (perencat bersandarkan cahaya), Kumpulan B (perangsang pertumbuhan anak benih), Kumpulan C (perencat pertumbuhan anak benih) dan Kumpulan D (perangsang bersandarkan cahaya). Sejumlah 16 bahan kimia didapati merangsang perubahan panjang hipokotil hanya dalam satu keadaan cahaya tertentu di mana 4-tert-butil-N'-(1-metilpirazolo[3,4-d]pirimidin-4-il)asibenzenkarboasimidamida dan 3-bromo-7-nitroindazol (3B7N) menggalakkan pemanjangan hipokotil dalam *Arabidopsis* jenis liar dalam cahaya biru dan tidak di bawah sinaran cahaya merah dan merah lampau. Analisis mutan *Arabidopsis cry1cry2* dan *phyAphyB* yang dirawat dengan 3B7N mengesahkan bahawa pemanjangan hipokotil yang diperhatikan dalam anak benih jenis liar adalah hasil daripada gangguan dalam fungsi CRY. Dengan pemprofailkan panjang hipokotil mutan CRY tunggal dan mutan berlebih meekspresor CRY yang dirawat dengan 3B7N, CRY1 telah dicadangkan sebagai fotoreseptor utama dalam mengawalatur fotomorfogenik *Arabidopsis* dalam cahaya biru. Profail kepanjangan hipokotil mutan-mutan COP1, HY5, HYH, LAF1 dan HFR1 yang dirawat dengan 3B7N mencadangkan gangguan antara interaksi protein CRY1 dan COP1 di hulu isyarat CRY menggalakkan pemanjangan hipokotil. Analisis profil ekspresi gen *Arabidopsis* jenis liar yang dirawat dengan 3B7N mengesahkan bahawa CRY mengawal transkripsi gen dan regulasi CRY terhadap pertumbuhan hipokotil dan melibatkan perubahan metabolik dalam *Arabidopsis*. Pemanjangan hipokotil dikawalatur CRY dalam *Arabidopsis* juga mendorong ekspresi gen faktor transkripsi dan melibatkan isyarat auksin dan gibberelin. Kesimpulannya, penyaringan bahan kimia LATCA pada *Arabidopsis* jenis liar mendapati satu bahan kimia yang khusus kepada cahaya biru iaitu 3B7N yang menggalakkan pemanjangan hipokotil dengan

merencat interaksi CRY1 dengan COP1.Gangguan fungsi CRY1 oleh 3B7N kemudian mengubah ekpresi gen cahaya biru yang diiktiraf, komponen isyarat hormon dan faktor transkripsi. 3B7N yang dikenalpasti daripada kajian ini boleh diaplikasikan kepada tumbuhan lain bagi mengkaji mekanisme cahaya biru dan kawalan genetik kriptokrom dalam pertumbuhan dan perkembangan tumbuhan.

IDENTIFICATION OF CHEMICALS AND CATALOGING OF GENES INVOLVED IN THE HYPOCOTYL ELONGATION OF *Arabidopsis* UNDER CONTINUOUS BLUE LIGHT

ABSTRACT

Plants have a remarkable ability to perceive and respond to various wavelengths of light and initiate the regulation of different cascades of light signaling and molecular components. While the perception of red light and the mechanisms of its signaling involving phytochromes (PHY) are largely known, the knowledge on blue light and the mechanisms of cryptochromes (CRYs) that control the seedling development is still not fully understood. This research aimed to identify the chemicals regulators of CRY-controlled photomorphogenesis and to catalog the genes involved in the chemical-induced hypocotyl elongation of *Arabidopsis*. The findings of this study contribute to a better dissection of cryptochrome roles in plant growth. The chemicals of The Library of Active Compounds on *Arabidopsis* (LATCA) were used and screened on *Arabidopsis* wild type (WT) Columbia (Col-0). The chemicals that stimulate hypocotyl elongation only in blue light were selected through comparison of the changes in hypocotyl length under various light conditions. The molecular mechanisms that promote hypocotyl elongation controlled by blue light were investigated through the characterization of hypocotyl response of loss-of-function mutants to chemicals. The changes on *Arabidopsis* gene expression induced by chemicals that stimulate hypocotyl elongation specifically in blue light was examined using microarray. Based on the response of cotyledon and hypocotyl of 6-day-old *Arabidopsis* WT seedlings treated with 3,680 chemicals, LATCA chemicals were clustered into four groups; Group A (light-dependent inhibitors),

Group B (seedling growth enhancers), Group C (seedling growth inhibitors) and Group D (light-dependent enhancers). A total of 16 chemicals were found to stimulate changes in hypocotyl length only in a specific light condition of which the 4-tert-butyl-N'-(1-methylpyrazolo[3,4-d]pyrimidin-4-yl)oxybenzenecarboximida-mide and 3-bromo-7-nitroindazole (3B7N) promote elongation of hypocotyl in WT *Arabidopsis* in blue light and not under irradiation of red and far-red light. Analysis of the *Arabidopsis* mutants *cry1cry2* and *phyAphyB* treated with 3B7N confirmed that the elongation of hypocotyl observed in WT seedling was a result of disruption in CRY function. Through profiling the hypocotyl response of single mutants and over-expressors of CRY treated with 3B7N, CRY1 was suggested as a prominent photoreceptor regulating *Arabidopsis* photomorphogenesis in blue light. The hypocotyl length profiles of mutants of COP1, HY5, HYH, LAF1 and HFR1 treated with 3B7N suggested the disruption of interaction between CRY1 and COP1 proteins at upstream of CRY signaling promotes hypocotyl elongation. Analysis of gene expression profiles of WT *Arabidopsis* treated with 3B7N confirmed that CRY controls gene transcription and CRYs regulation of hypocotyl growth involves a metabolic change in *Arabidopsis*. CRYs-mediated hypocotyl elongation in *Arabidopsis* induces expression of many transcription factor genes and also involves the auxin and gibberellin signaling. In conclusion, LATCA chemical screen on WT *Arabidopsis* identified a blue light-specific chemical, 3B7N that promoted hypocotyl elongation by inhibiting interaction of CRY1 with COP1. The disruption of CRY1 function by 3B7N then altered expression of the recognized blue light genes, hormone signaling components and transcription factors. The identified 3B7N from this study can be applied for application on other plants to study the blue-light

mechanism and genetic control of CRYs in the growth and development of plant species.

CHAPTER 1

INTRODUCTION

1.1 Introduction and Research Objectives

Light is an important factor for the successful growth and development of plants (Fankhauser & Chory, 1997; Jiao et al., 2007; Kami et al., 2010). Plants depend on light for photosynthesis. Germination does not occur in complete darkness and occurs only when plants sense low amount of light. Below ground level, germinated seedlings elongate the hypocotyl to reach for light and the cotyledon is closed and yellowish in colour as the absence of light blocks chlorophyll synthesis (Josse & Halliday, 2008). Above ground level, the seedling senses light and undergoes photomorphogenesis, a development where the hypocotyl elongation ceases and cotyledon starts to open and the colour changes to green as the chloroplast begins developing (Arsovski et al., 2012). This developmental transitions from a dark-grown seedling to a light-grown seedling was termed de-etiolation (Arsovski et al., 2012).

Light is made up of wavelengths of light and different wavelengths give different light colour. Plants often exploit the red and far-red light to determine germination (Tiansawat & Dalling, 2013). Red light induces seed germination while far-red light slows down germination process (Sullivan & Deng, 2003). Besides red and far-red light, plants sense blue light. Blue light controls many physiological responses in plant such as hypocotyl elongation, stomata opening, flowering time and synthesis of the anthocyanin (Christie & Briggs, 2001). Plants are equipped with

photosensory proteins that function in receiving different types of light wavelength to initiate the respective signalling pathway for appropriate regulation of physiological and morphological responses (Galvão & Fankhauser, 2015). Photoreceptors regulate plant development, morphogenesis and physiology to respond to the changes in light intensity, quality, direction and duration of light they sense (Christie et al., 2015).

In *Arabidopsis,* de-etiolation is controlled by the blue light-sensing, cryptochromes (CRYs) and red or far-red light-sensing, phytochromes (PHYs) (Kong & Okajima, 2016). Both CRYs and PHYs play crucial roles in light-mediated regulation of seedling development through transcriptional network (Chen et al., 2004). The *Arabidopsis* genome encodes five PHY (PHYA-PHYE) and the PHYA and PHYB play most important roles in light-dependent development in plant and their mechanisms of actions are largely investigated (Franklin & Quail, 2010; Strasser et al., 2010). PHYs are unique photoreceptors as they exist in two interconvertible forms, the inactive red-absorbing Pr isoform and the active far-red-absorbing Pfr isoform (Su et al., 2017). Red light induces a conformational change and transforms Pr into biologically active Pfr and mediates responses such as seed germination and de-etiolation (Li et al., 2011). Irradiation of far-red light converts Pfr into the inactive Pr form. In the absence of light, PHYs accumulate in Pr forn in cytosol, and upon red light absorption, PHYs are translocated into the nucleus and initiate transcription of genes that mediate light-dependent responses (Wang & Deng, 2002; Josse & Halliday, 2008). PHYB is the main receptor of red light while PHYA is the receptor of far-red light. When the ratio of red light to far-red is high, PHYB regulates the inhibition of the petiole and internode elongation (Martinez-Garcia et al., 2014). Under dense canopy whereby the amount of red light is low and far-red

light is high, PHYB is inactive and PHYA antagonizes the loss of PHYB activity resulting in modest hypocotyl elongation (Kami et al., 2010).

Arabidopsis contains several types of blue-light photoreceptors. FLAVIN BINDING KELCH REPEAT F-BOX 1 (FKF1) and ZEITLUPE (ZTL) are photoreceptors for the control of circadian rhythm and photoperiodic flowering (Nelson et al., 2000; Somers et al., 2000; Ito et al., 2012). Phototropin 1 (PHOT1) and phototropin 2 (PHOT2) are photoreceptors controlling phototropism and chloroplast movements while cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2) are photoreceptors controlling hypocotyl elongation, germination, stomata opening and flowering time (Liu et al., 2016). The flowering time in *Arabidopsis* depends on the expression of FLOWERING LOCUS T (*FT*) gene regulated by CONSTANS (CO) protein (Suárez-López et al., 2001; Valverde et al., 2004). Mutation of the FKF1 and ZTL proteins suppressed the expression of *FT* gene and delayed flowering in *Arabidopsis* (Nelson et al., 2000; Imaizumi et al., 2003). The ZTL plays a crucial role in circadian clock progression. In dark, ZTL interacts with TIMING OF CAB EXPRESSION 1 (TOC1) and mediates degradation of TOC1 through 26S proteosome pathway (Fujiwara et al., 2008). The change of the TOC1 level affects expression of clock genes such as LATE ELONGATED HYPOCOTYL (*LHY*), CIRCADIAN CLOCK-ASSOCIATED 1 (*CCA1*), PSEUDO-RESPONSE REGULATORS 9 (*PRR9*) and *PRR7* (Greenham & McClung, 2015).

CRYs serve a distinct yet overlapping function in regulating photomorphogenic responses and photoperiodic flowering (Liu et al., 2016). CRY1 is mainly involved in de-etiolation while the CRY2 is the predominant blue light

receptor regulating photoperiodic flowering (Liu et al., 2011). While the perception of red light and the mechanisms of its signalling involving PHYs are largely known, knowledge of the mechanisms of blue light signalling is still limited. PHYB mediates low light fluence-induced red or far-red-reversible seed germination and hypocotyl elongation inhibition under continuous red light (Shinomura et al., 1996). Recently, two novel inhibitors of cryptochromes, BLUE-LIGHT INHIBITOR OF CRYPTOCHROMES 1 (BIC1) and BIC2 were found to interfere the blue light-dependent dimerization of CRY2 (Wang et al., 2016). However, there is not much knowledge about the reverse action and mechanism of CRY1 and how transcriptional network changes when the blue light-dependent activity of CRY1 is inhibited.

Chemicals have many advantages over proteins or nucleic acid-based agents for studying biological systems. The application of chemicals had played a crucial role and is notably advantageous in dissecting complex mechanism over classical genetic approaches. Chemical genetic approach circumvents the problems of lethality and redundancy (yielding no observable change in phenotype) of classical method which gene functions are revealed by linking mutation (genotype) to the phenotype (Sikora et al., 2011; Oladosua et al., 2016). Chemical affects active sites of a protein target or whole class of protein targets and the effect by chemicals that are instantaneous, tuneable and even reversible allows wider application and deeper investigation into biological processes (Raikhel & Pirrung, 2005; Hicks & Raikhel, 2009, 2012).

Recently, plant biologists have begun to use chemicals for basic plant research to understand cell wall biosynthesis, hormone biosynthesis and signalling,

pathogenesis and endomembrane trafficking (Armstrong et al., 2004; Yoneda et al., 2007, 2010; Meesters et al., 2014). Chemical screens to gain insight into hormone signal transduction had led to (a) the identification of SIRTINOL RESISTANT 1 (SIR1), a new protein which is an upstream regulator of auxin signalling (Zhao et al., 2003), (b) discovery of a new mode of cross-talk between ethylene and brassinosteroid (BR) through analyses of mutants response to brassinopride by screening chemicals which inhibit the expression of brassinosteroid gene (Gendron et al., 2008) and (c) precise understanding of the multitude plant responses to bioactive jasmonates (JA) (Turner et al., 2002; Meesters et al., 2014).

Plants have an immune system to protect them from diseases. Plant activators can be used to protect plants from diseases by activating their immune system. Salicylic acid (SA) is an important metabolite that activates the plant immune system (Noutoshi et al., 2012a). A chemical screen for enhancer of SA discovered imprimatin C1 which functions as a weak analogue of SA and activates the expression of defence-related genes (Noutoshi et al., 2012b). A chemical screen on *Arabidopsis* liquid culture for *Pseudomonas* inhibitors had led to identification of plant activators, sulfameter, sulfamethoxypyridazine, sulfabenzamide, and sulfachloropyridazine belonging to the sulfonamide group that induce *Pseudomonas*-induced cell death (Schreiber et al., 2008; Noutoshi et al., 2012a).

The plant endomembrane system is functionally complex and composed of organelles, vesicular compartments and peripheral membrane components. Endomembrane trafficking has a key role for ensuring homeostasis (Drakakaki et al., 2009; Mishev et al., 2013). A high-throughput chemical screen focusing on the rapid

recycling of plasma membrane markers identified specific inhibitors function in different path of *Arabidopsis* protein trafficking. It is further revealed that endosidin 3 can alter Rho-of-plants (ROP)-mediated signalling involved in cell polarity, endosidin 5 treatment interferes with endomembrane-trafficking pathways to the vacuole, while endosidin 7 obstructs *Arabidopsis* cell-plate maturation (Drakakaki et al., 2011). The chemicals targeting different proteins or pathways for different responses and function are useful to assist the detailed investigation of endomembrane trafficking.

In this study, forward chemical genetic was applied to investigate the action and mechanism governing the inhibition of de-etiolation controlled by cryptochrome under blue light. A chemical screen was conducted to select light specific inhibitors. The Library of Active Compounds on *Arabidopsis* (LATCA) library consisting chemicals that are effective in yeast or *Arabidopsis* was used. Chemical screening was conducted on wild-type (WT) *Arabidopsis* and chemical effect was evaluated based on seedling morphology. Following this, 3-bromo-7-nitroindazole (3B7N) which induced long hypocotyl in WT seedlings with blue light was further investigated. The 3B7N was tested on loss-of-function mutants and hypocotyl length of mutants and over-expressors were evaluated to dissect the chemical inhibitory effect in blue light signalling pathway. Photoreceptors affect multiple molecular mechanisms including transcriptional activation of genes and transcription factors. The third part of this study involves investigation on *Arabidopsis* gene expression by specific blue light. This research is aimed to achieve the following objectives with the experiment workflow explained above.

(a) To establish a chemical screening method for light-dependent inhibitors.

(b) To identify specific chemicals inducing hypocotyl elongation under blue light.

(c) To characterize 3B7N regulation of hypocotyl elongation in *Arabidopsis* using loss-of-function mutants.

(d) To analyze the expression of genes involved in 3B7N-induced hypocotyl elongation of *Arabidopsis* in blue light using microarray.

CHAPTER 2

LITERATURE REVIEW

2.1 *Arabidopsis thaliana* (*Arabidopsis)*

Arabidopsis thaliana (*Arabidopsis*), also known as thale grass, is a member of the family Brassicaceae which grows low on the ground and produces clusters of small white flowers (The *Arabidopsis* Genome Initiative, 2000). Eventhough this plant is not of major agronomic significance, *Arabidopsis* offers important advantages for basic research in plant genetics and molecular biology (Koornneef & Meinke, 2010). *Arabidopsis* was the first plant which the genome to be completely sequenced. The *Arabidopsis* has five chromosomes with a genome size of about 150 Mbp (The *Arabidopsis* Genome Initiative, 2000).

Arabidopsis is the model plant for studies of growth and development because it has a relatively short life cycle. The germination phase including seed imbibition, radicle emergence and full developed of cotyledon and hypocotyl takes five to seven days (Figure 2.1). The leaf development phase from cotyledons to growth of 14 rosette leaves takes two weeks. The process from initiation of rosette growth to inflorescence emergence takes about 10 days and from inflorescence emergence to silique ripening takes another two to three weeks. Thus, *Arabidopsis* complete a full cycle from seed germination to full adult plant with seed maturation in a period of two months (Boyes et al., 2001). Adult *Arabidopsis* plant measured a height of 20 cm. The small size *Arabidopsis* that limited the requirement for growth

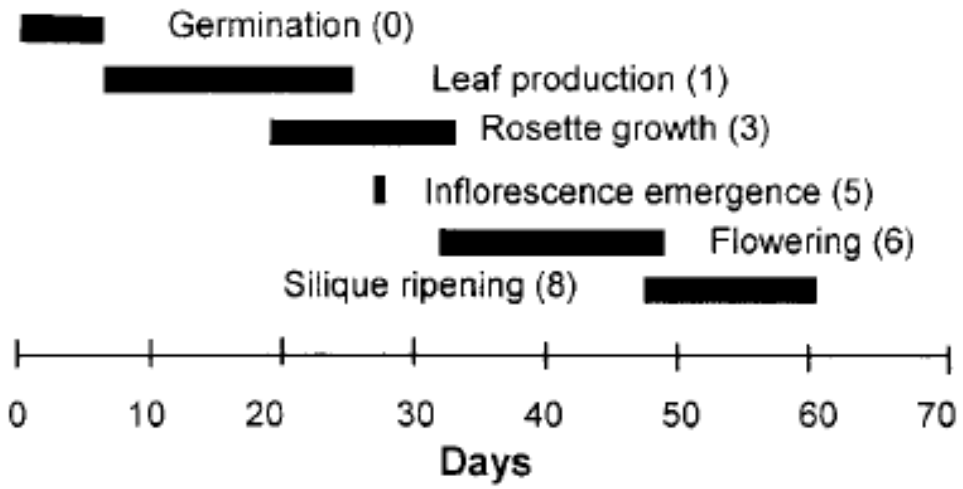

Figure 2.1 Schematic timeline growth of the chronological progression of principal *Arabidopsis* growth stages. Horizontal bars indicate the period during wild type Columbia-0 Col-0 plant development and the numbers in parentheses correspond to principal growth stage. The growth stages are (0) germination stage, between day-0 and day-3; (1) leaf production stage, between day-5 and day-25; (3) rosette growth stage, between day-19 and day-29; (5) inflorescence emergence stage, starting at day-26; (6) flowering stage, between day-32 and day-48; (8) silique ripening, starting at day-48 (Boyes et al., 2001).

facilities is an advantage for high density propagation in green house or in culture room (Boyes et al., 2001).

Transformation, a process of introducing plasmid-carrying gene construct into cell is a core research tool for functional analysis of genes and is a useful tool for cultivar improvement (Rao et al., 2009). Transformation using *Agrobacterium tumefaciens* is well established and is widely applied in *Arabidopsis* (Meinke et al., 1998). Efficient and easy transformation methods such as seed transformation or intact plant transformation by 'floral dip' have been developed for *Arabidopsis* allowing easy genetic manipulation (Clough et al., 1998; Meinke et al., 1998; Gelvin et al., 2003).

Introduction of the large transfer deoxyribonucleic acid (T-DNA) sequence into plant genome which create mutation generated mutant lines (Gelvin et al., 2003). Insertion of T-DNA is random and insert of T-DNA into the open-reading-frame orpromoter of a gene often disrupts the gene function (Gelvin et al., 2003). Phenotypic assessment of mutants and sequence determined of insertion site, tag gene to its function (Malley & Ecker, 2010). T-DNA collection has been a good resource and there are about 330, 000 *Arabidopsis* T-DNA insertion lines available at web portals including The *Arabidopsis* Information Resource (TAIR) (https://www.arabidopsis.org/), Kōlner *Arabidopsis* T-DNA lines (GABI-Kat) (https://www.gabi-kat.de/) and Salk T-DNA Express (http://signal.salk.edu/cgi-bin/tdnaexpress) (Malley & Ecker, 2010; Kleinboelting et al., 2012). Besides the mutants collection, *Arabidopsis* full-length complementary DNA (cDNA) sequences

have been isolated and the full-length clones are available from the RIKEN Bioresource Center (http://epd.brc.riken.jp/en/pdna/rafl_clones) (Seki et al., 2002).

The *Arabidopsis* mutants are a valuable resource and are largely applied to discover useful genes. Recently, screening thousands of *Arabidopsis* mutants for resistant and susceptible mutants in high heavy metal condition reveal useful genes for phytoremedition. Most of the mutations are genes which play roles in transport, protein modification and signalling as well as the ribonucleic acid (RNA) metabolism (Sanz-Fernández et al., 2017). These mutants with different in growth and tolerance to heavy metals are practical for investigation and understanding the mechanisms involved in plant heavy-metal perception. During cytokinesis in plant cell, the formation of a new cell plate is accomplished by phragmoplast (Li et al., 2015). A microscope-based screen of *Arabidopsis* mutants for mutations affecting cell division identified a few mutants that form abnormally enlarged cells with incomplete cell walls. Investigation of these mutants reveals two genes, *Arabidopsis* Fused kinase and kinesin-5 with new function in the formation of the phragmoplast and the cell plate in the embryo (Stewart-Gillmor et al., 2016).

Arabidopsis plants carrying disruption in the photoreceptorgenes were among the first to provide evidence for the involvement of photoreceptors in light perception of plants. There are thirteen characterized sensory receptors perceiving different light wavelengths in *Arabidopsis*. There are five phytochromes copies in *Arabidopsis* and roles of each phytochromes on promoting germination, seedling development and control of flowering in temperature-dependent manner are widely reported (Sánchez-Lamas et al., 2016). The blue light receptor, cryptochromes, was first identified by

Ahmad and Cashmore (1993) from *Arabidopsis* mutant displaying elongated hypocotyl. Following this discovery, cryptochromes were also found in other plant species including white mustard, fern, mosses, tomato, rice and barley (Batschauer, 1993; Kanegae & Wada, 1998; Perrotta et al., 2000; Perrotta et al., 2001; Imaizumi et al., 2002; Matsumoto et al., 2003). Over the past two decades, many proteins function in light signaling are discovered in *Arabidopsis* and the interactions between light signaling proteins in various conditions are investigated.

2.2 The Role of Light in the Life Cycle of Plants

Sunlight is an electromagnetic wave, which acts as the energy source for plants. Unlike animals, plants are unable to move away from an unfavorable environmental stimulus. Plants maximize their chances of survival by optimizing their developmental patterns in a way that adapt to the changing environment. Of all the environmental cues, lighting factor is probably the most important signal (von Arnim & Deng, 1996; Howell, 1998). Plants perceive the presence of light of their surroundings including the light spectral quality (wavelength), intensity, direction and duration of light exposure in order to optimize their growth (Fankhauser & Chory, 1997; Sullivan & Deng, 2003). Plants perceive sunlight and produce energy for themselves through photosynthesis (Folta & Childers, 2008). Besides serving as a source of energy, light also initiates and controls many aspects of plant development. These include seed germination, photomorphogenesis, leaf development, shade avoidance and changes in flowering time (Wang & Deng, 2003; Chen et al., 2004; Jiao et al., 2007).

2.3 Skotomorphogenesis and Photomorphogenesis

The seedling is a transition from embryonic to postembryonic development and light is a major factor influencing the route of seedling development. Seeds that germinate below ground level undergo skotomorphogenesis, a form of development in the dark (Josse & Halliday, 2008). Dark-grown (etiolated) seedlings have an elongated hypocotyl that pushes the closed cotyledons upwards through the soil. The absence of light results in an apical hook-like, closed, and pale yellowish colored cotyledon (Figure 2.2A). When a seedling emerges to the surface and is exposed to light, the seedling undergoes photomorphogenesis and seedlings begin to de-etiolate (Sullivan & Deng, 2003). Under light conditions, a seedling emerges from the seed coat, consisting of a hypocotyl with an apical hook, two small folded cotyledons, and a short main root. The hypocotyl of seedling ceases rapid elongation and becomes thicker which is attributed primarily to hydration. The cotyledons open and expand by cell division and cell expansion followed by chlorophyll accumulation and greening (Figure 2.2B).

Photomorphogenesis or de-etiolation, the transition from a heterotrophic darkness-dwelling state to an autotrophic light-dwelling state offers a great opportunity to investigate light signal transduction mechanism in development process of plants (von Arnim & Deng, 1996). Investigations of de-etiolation and etiolation state of *Arabidopsis* in different light qualities, including red, far-red and blue lights identified approximately one-third of the genome with differential expression (Ma et al., 2001). In addition, significant fluctuation in the expression of a large number of genes at early period of light induction provides the idea that a small amount of light irradiation can trigger different changes to the light-regulated genes

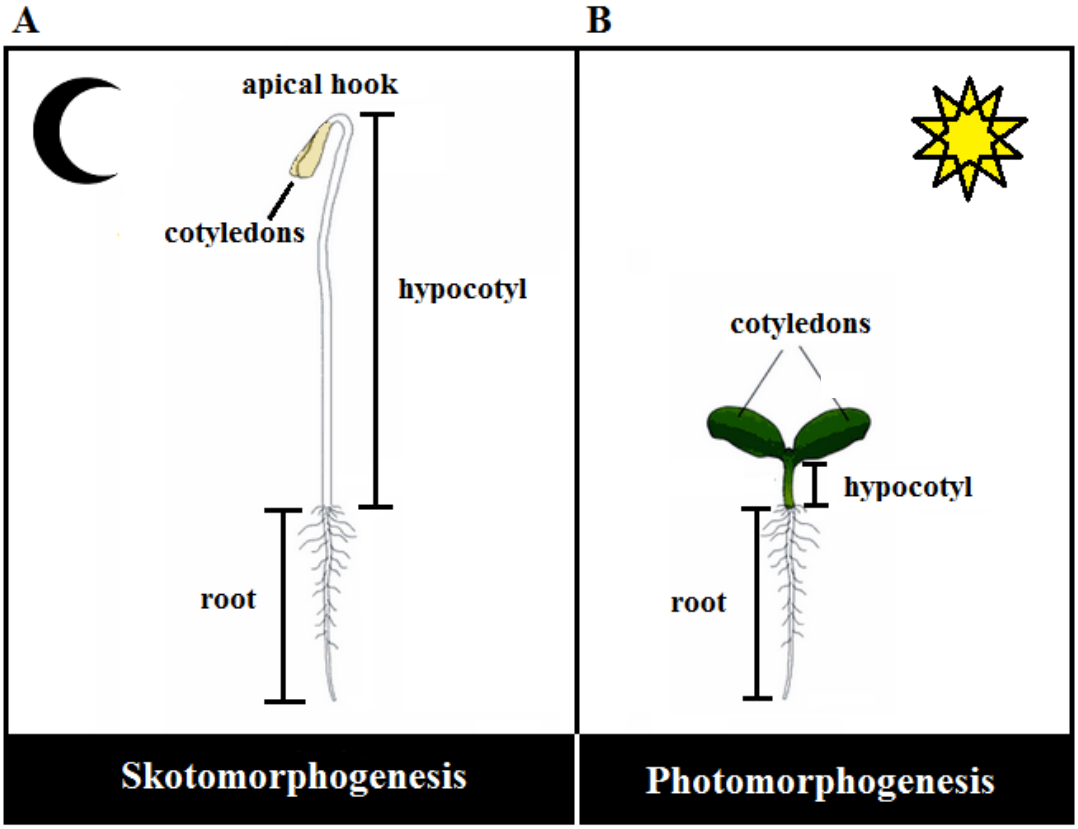

Figure 2.2 Comparison of *Arabidopsis* seedlings grown in the dark and in the presence of light. (A) Etiolated seedlings grown in dark have long hypocotyl, apical hook (at early stages) and unexpanded, closed pale cotyledons. (B) Light-grown, de-etiolated seedlings have short hypocotyl and expanded green cotyledons (modified from Howell, 1998).

(Ma et al., 2001). In many investigations on light made changes to gene expression in plants,the expression of glyceraldehydes-3-phosphate dehydrogenases (GADPH), chalcone synthase (CHS), phenylalanine ammonia-lyase (PAL), phytochrome A, protochlorophyllideoxidoreductase (POR), asparagine synthetase (AS) and tubulin beta-1 (TUBB1) genes are constantly expressed in presence of light (Schroder et al., 1979; Apel, 1981; Cerff & Kloppstech, 1982; Bruce et al., 1989; Batschauer et al., 1991; Leu et al., 1995; Shi et al., 1997). Examination of the expression changes provides important information towards understanding the mechanism of light signaling controls of plant growth anddevelopment (Jiao et al., 2003). There are at least 26 pathways involved in *Arabidopsis* seedling growth in light conditions. Pathways of those maintaining plant photosynthesis, starch, protein and amino acid biosynthesis, cell wall synthesis, photorespiration and the phenylpropanoid pathway are controlled by light.

2.3.1 Etiolation

Etiolation is a process occurs in plants grow in environment with low light or complete absence of light (Arsovski et al., 2012). During etiolation, changes including development of apical hook, weakening cell wall of stems, elongation of stems and leaves, elongation of internodes and clorosis, and the reduction or loss of the normal green coloration of leaves, take place in plants. Prolonged darkness result in retardation of growth, senescence and even death of plants (Ishizaki et al., 2005). Light, temperature and phytohormone such as auxin, ethylene and BR play important roles in the etiolation of plants (Clouse et al., 2001; De Grauwe et al., 2005; Garmash et al., 2015; Humplík et al., 2015; Boex-Fontvieille et al., 2016). Light negatively influences hypocotyl elongation by activating the PHYs. Photoactivated PHYs

induces degradation of PHYTOCHROME-INTERACTING FACTOR (PIF) via the ubiquitin-proteasome system. In dark or low ratio of red to far red light, PIF3, PIF4 and PIF5 proteins are stable (Lorrain et al., 2008). The abundance of PIFs protein are able to activate auxin biosynthesis genes and promotes hypocotyl elongation (Lorrain et al., 2008).

Auxin is required for apical hook formation and cell elongation in plant (Lehman et al., 1996; Ge et al., 2017). Exogenous applications of auxin or mutation of auxin-synthesis genes causes defects in apicalhook formation (Schwark & Schierle, 1992). Similarly, plants treated with naphthylphthalamic acid, the chemical that blocks polar auxin transport resulting in plants with defects in hook formation (Lehman et al., 1996). In *Arabidopsis*, auxin-induced hypocotyl elongation of etiolatedseedlings is mediated by activation of proton-pumping ATPase via phosphorylation (Takahasi et al., 2012). Besides light intensity, high temperature (29 °C) also promotes auxin-mediated hypocotyl elongation in *Arabidopsis* (Franklin et al., 2011; Maharjan & Choe, 2011; Zheng et al., 2016; Ge et al., 2017). High temperature promotion of hypocotyl elongation in *Arabidopsis* involves both the auxin and BR. High temperature-dependent synthesis of auxin stimulates BR biosynthesis through induce expression of *DWARF4* in the shoot and root tips (Maharjan & Choe, 2011).

There are highly overlapping genes found regulated by auxin and BR and both hormones are known to induce cell elongation (Kang et al., 2001; Zhou et al., 2013). Exogenous BR treatment increases the hypocotyl length and the enhanced BR signaling induces the differential growth of etiolated hypocotyls (Nemhauser et al.,

2004; Zhou et al., 2013). Molecular and biochemical investigation of BR involvement in etiolation of plants found the interaction of light-repressible small G protein with cytochrome P450 C-2 hydroxylase protein is responsible for the etiolation and de-etiolation transition (Kang et al., 2001). The cytochrome P450 is predominantly expressed in etiolated pea and overexpression of the gene promoted hypocotyl growth even in the light (Kang et al., 2001; Nakamura et al., 2005).

Ethylene is also found necessary for the maintenance of the hook in etiolated *Arabidopsis* and ethylene stimulates hypocotyl elongation in the light (Raz & Ecker, 1999; Vriezen et al., 2004). Ethylene response in the control of hypocotyl growth depends on CRY1 action (Folta et al., 2003; Vandenbussche et al., 2007). Concurrent treatment of c*ry1* seedlings with prohexadione calcium and gibberellin (GA) reversed the long-hypocotyl phenotype typical of *cry1* mutant. Ethylene-mediated enhancement of apical hook formation and hypocotyl elongation in etiolated *Arabidopsis* seedlings is GA dependent but not mediated by GA signaling (Vriezen et al., 2004; Vandenbussche et al., 2007). The ethylene precursor 1-aminocyclopropane-1-carboxylate inhibits cell elongation in the apical hook by inhibition of GA signaling (Vriezen et al., 2004). In addition, CRY1-dependent regulation of hypocotyl growth encoded a large numbers GA biosynthesis genes and auxin response factors (Folta et al., 2003). Crosstalk exists between auxin and ethylene for mediating hypocotyl elongation as ethylene is involves in auxin transport (Swarup et al., 2007; Zádníková et al., 2010).

Hypocotyl elongation is a complex developmental process regulated by many factors. Besides phytohormones, xyloglucan endotransglucosylase, expansins,

polygalacturonases, pectin methylesterases, peroxidases and serine proteases are abundantly expressed in hypocotyl elongation process in etiolated *Arabidopsis* (Irshad et al., 2008). In dark-grown *Arabidopsis* seedlings, Kunitz-protease inhibitor 1 is presence in the apical hook and elongated hypocotyl region and inhibits the cysteine protease RESPONSIVE TO DESICCATION 21 (Jiménez, et al., 2007; Boex-Fontvieille et al., 2016). The actions of the various plant hormones are an overlapping of processes that are regulated through the activity of the sensory photoreceptors systems which is dependent on light quality and intensity.

2.4 Plant Photoreceptors

In order to monitor the changes in a light environment, plants have evolved a series of photoreceptors which absorption properties match the different spectrums of incoming light (Briggs & Olney, 2001). Photoreceptors are considered as such if upon photon absorption, they are able to deliver a signal to downstream components. Plant membranes are transparent to light andmost photoreceptors are cytoplasmic and water soluble (Moglich et al., 2010).

In *Arabidopsis*, four classes of wavelength specific photoreceptors are present. They are: (a) phytochromes (PHYs) which respond to red and far-red lights of between 600 to 750 nm (Quail et al., 1995; Chory et al., 1996); (b) cryptochromes that are (CRYs) the blue light absorbing photoreceptors responding to wavelengths of between 350 to 500 nm (Ahmad & Cashmore, 1997; Lin, 2000); (c) phototropins (PHOTs) which are the blue light and UV-A light absorbing wavelengths between 320 to 500 nm (Briggs & Christie, 2002; Christie, 2007) and (d) UV RESISTANCE LOCUS 8 (UVR8) which are the ultraviolet-B (UV-8) light absorbing photoreceptors