

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Photoreceptor cross-talk in UV-B photomorphogenesis in
tomato (*Solanum lycopersicum*): Screening through
phytochrome and cryptochrome mutants**

A thesis presented in partial fulfilment of the requirements for the degree

of

Master of Science in Plant Biology

At Massey University, Palmerston North, New Zealand



Ivie V. S. Pabellon

2017

Abstract

Plant photoreceptors detect changes in the light environment and induce differential gene expression, resulting in the appropriate physiological and morphological responses. Under full sunlight, phytochromes, cryptochromes and the UV-B photoreceptor, UVR8 (UV-B RESISTANCE LOCUS 8), destabilize PHYTOCHROME INTERACTING FACTORS (PIFs) to inhibit elongation. PIFs are transcription factors that inhibit light-regulated genes, including auxin-related genes involved in cell elongation. In the shaded environment, the reduction in the spectral composition detected by the photoreceptors results in the activation of elongation and PIF activity. However, recent studies have shown that low levels of UV-B can still inhibit the elongation under shade.

Most photobiology studies that investigated plant responses to shade have concentrated on the model species, *Arabidopsis thaliana*. In contrast, *Solanum lycopersicum* (tomato) is another model system, but few studies have investigated plant responses to shade in tomato due to its sympodial architecture and presence of internodes which *A. thaliana* lacks. In this study, phytochrome and cryptochrome tomato mutants were exposed to low levels of UV-B under photosynthetically active radiation (PAR) as background light to investigate the possible cross-talk between these photoreceptors and the UV-B photoreceptor of tomato in regulating hypocotyl or internode elongation. Out of all the multiple phytochrome and one cryptochrome mutants, *phyAphyB2* mutant exhibited an impaired UV-B inhibition of internode elongation after three days of UV-B treatment. End-point PCR on the gene expression of PIF4 together with two UV-B responsive genes and genes involved in the catabolism of active gibberellin could not explain the impaired response of *phyAphyB2*. Nevertheless, physiological measurements indicate that phyA and phyB2 of tomato may be acting redundantly in mediating the UV-B induced inhibition of internode.

Acknowledgements

I would like to express my gratitude to all the people who have helped me throughout this thesis. To my supervisors: Dr. Huub Kerckhoffs and Dr. Jason Wargent, thank you for giving me the opportunity to work with you. Thank you for the support, the motivation and your advices that have kept me to move forward. I have learnt so much from both of you-thank you.

Thank you to Steve, Lindsay and Lesley at the Plant Growth Unit, for helping me in maintaining the growth chambers and looking after my plants. To Chris Rawlingson and Sunmeet, thank you for helping me with the equipment that I need for my experiments. Thank you also to Dr. Paul Dijkwel and his group for letting me their laboratory for all my PCR work. Also, thank you to my photobiology group for the support throughout my thesis especially to Konstancija, thank you for helping me collect my samples.

I would like to also thank BioLumic Ltd. for letting me use their LED lights throughout the span of my experiments. And to the BioLumic staffs: Hangfeng, Monica and Claudia for the advices and technical support.

Thank you to the Helen E. Akers scholarship for the support during my last year of Masters.

To my friend Rixta, thank you for helping me with my PCR experiments and for offering to grammatically check my thesis. Also thank you for your words of wisdom that have kept me motivated until the end of writing this thesis.

Lastly, thank you to my parents, Alice and Francisco; and to my sisters, Myca and Joy, for the constant support, love, sacrifice and patience throughout my postgraduate journey.

Table of Contents

Abstract	i
Acknowledgements	iii
Table of Contents	vi
List of Figures	xi
List of Tables	xii
Abbreviations	xiii
1.0 Introduction	1
1.1 Light detected by plants	1
1.2 <i>Arabidopsis</i>	2
1.2.1 Phytochromes	3
1.2.1.1 Types of phytochromes	4
1.2.1.2 Phytochrome response modes	5
1.2.2 Cryptochromes	6
1.2.3 UVR8	7
1.3 Light and plant development	8
1.3.1 Stages of plant development and strategies under light limiting conditions	8
1.3.2 Light Signals that activate the Shade Avoidance Responses	9
1.3.3 Roles of phytochromes, cryptochromes and UVR8 in Shade Avoidance	11
1.3.3.1 Phytochromes mediate responses to low R:FR	11
1.3.3.2 Cryptochromes and phytochromes induce shade avoidance elongation response under low B light	12
1.3.3.3 UV-B inhibit growth response in shade	13
1.3.4 Cost of Shade Avoidance Response	13

1.3.5	Molecular mechanism of photomorphogenesis and shade avoidance response	14
1.4	Significance of UV-B studies	18
1.4.1	Ozone depletion motivated UV-B studies	18
1.4.2	UV-B provide photoprotection and defence	20
1.5	Tomato as another model system	21
1.5.1	Why study tomato plant responses under UV-B?	21
1.5.1.1	Tomato: Economic importance	21
1.5.1.2	Tomato architecture: plant model for shade avoidance	22
1.5.2	Photoreceptors of Tomato	23
1.5.2.1	Characterization of tomato phytochromes	23
1.5.2.1.1	Tomato phytochrome A (far-red insensitive, <i>fri</i>)	23
1.5.2.1.2	Tomato phytochrome B1 (temporarily red light insensitive, <i>tri</i>) and phytochrome B2	24
1.5.2.1.3	Roles of phytochrome A, B1, B2 in shade avoidance response	25
1.5.2.1.4	Cryptochromes	26
1.5.2.1.5	UV-B photoreceptor	26
1.6	Project Aims	27
1.6.1	Questions to be answered	28
1.6.2	Hypothesis	28
2.0	Materials and Methods	29
2.1	Plant Material and growing conditions	29
2.2	Transplanting and Allocation	31
2.3	Light Treatments	32
2.4	Measurements	34

2.5 End-Point PCR	35
2.5.1 Sample preparation and RNA extraction	35
2.5.2 Genomic DNA extraction	35
2.5.3 DNaseI treatment of RNA samples and cDNA synthesis	37
2.6 General functions of genes of interests	39
2.6.1 Tomato PHYTOCHROME INTERACTING FACTOR 4 (<i>SIPIF4</i>)	39
2.6.2 Tomato long hypocotyl 5 (<i>LeHY5</i>)	41
2.6.3 Tomato GIBBERELLINE 2 OXIDASE 2 (<i>Ga2ox2</i>)	41
2.6.4 Tomato Chalcone Synthase 1 (<i>CHS1</i>)	42
3.0 Results	43
3.1 Seed germination	43
3.2 Screening using low fluence UV-B	44
3.3 Increase in WT hypocotyl elongation rate is not due to shading	46
3.4 Low fluence UV-B is unable to inhibit the internode of <i>phyAphyB2</i>	48
3.4.1 UV-B inhibition responses of the hypocotyl and internode may be age-dependent	50
3.5 UV-B treatment is more effective in inhibiting hypocotyl when applied in the morning	52
3.6 PCR troubleshooting using housekeeping genes: TUBULIN and ACTIN	53
3.6.1 TUBULIN primers are not annealing to tomato tubulin	53
3.6.2 ACTIN primers are more consistent in amplifying tomato <i>ACTIN</i>	57
3.7 UV-B increases expression of light regulated genes of UV-B treated tomatoes	58
4.0 Discussion	60
4.1 Germination of tomato phytochrome and cryptochrome mutants	60

4.2 Increase in WT hypocotyl elongation may be due to UV-B entrainment on the plant's circadian clock	61
4.3 Phytochrome A and B2 activity may be involved interacting with the UV-B photoreceptor of tomatoes in regulating internode elongation under UV-B	62
4.4 Tomato LONG HYPOCOTYL 5 (<i>LeHY5</i>) and Chalcone Synthase 1 (<i>CHS1</i>) as UV-B-responsive marker gene	65
4.5 Dose-dependent upregulation of tomato <i>SIP4</i> after four hours of UV-B.	66
4.6 Gibberellic 2 oxidase2 (<i>Ga2ox2</i>) upregulation typical response to UV-B inhibition of cell elongation	67
5.0 Conclusion	68
Appendices	
APPENDIX A: Table 1: RNA concentrations of 16 samples measured using Nanodrop.	70
APPENDIX B: Table 1: Primers used in endpoint PCR.	71
Table 2: Annealing temperatures used for amplification of target genes during PCR.	71
APPENDIX C: Table 1: PCR program used to amplify target genes.	72
APPENDIX D: Figure 1: Summary of PCR optimization.	73
References	74

List of Figures

Figure 1.1: Electromagnetic spectrum detected by plant photoreceptors	3
Figure 1.2: Photoequilibrium of the inactive (Pr) and active (Pfr) form of phytochromes	4
Figure 1.3: Photomorphogenic response of plants under high R:FR ratio inhibit growth and low R:FR induce stem elongation	9
Figure 1.4: Proposed model illustrating molecular interaction between phytochromes and UVR8 in environments where there are no competitions and presence of neighbouring plant competitors.	15
Figure 1.5: Summary of photoreceptor signaling under full sunlight.	18
Figure 1.6: Architectural difference between wild-type Arabidopsis and tomato.	23
Figure 2.1: Fluorescent tubes light spectrum measured using the Optronics 756 spectroradiometer.	30
Figure 2.2: Plants allocated in two conditions: crowded (3 days after treatment) and non-crowded condition (2 days before treatment)	31
Figure 2.3: First treatment schedule.	32
Figure 2.4: Second treatment schedule.	33
Figure 2.5: Developmental stage of WT (and other mutants) at 14 DAS on the day of treatment.	34
Figure 3.1: Developmental stage at which plants were treated. Plants (13-15 DAS) treated had two true leaves emerging. (Image above is 14 DAS WT under PAR light.)	43
Figure 3.2: Tomato wild-type, phytochrome and cryptochrome mutants' relative hypocotyl and internode growth rate after treatment of PAR and PAR+UV-B for three days.	45

Figure 3.3: Hypocotyl and internode growth rate of WT exposed to two light conditions: PAR and PAR + UV-B for 3 days.	46
Figure 3.4: Relative hypocotyl and internode growth rate of phytochrome and cryptochrome mutants after exposure to low dose of UV-B for three days.	48
Figure 3.5: Percentage UV-B inhibition of internode elongation after 3 days of PAR + UV-B treatment.	49
Figure 3.6 Developmental check of hypocotyl and internode growth of WT, phyAphyB1, phyAphyB2 and cry1 of tomatoes throughout the experimental period.	50
Figure 3.7 End-of-day-treatment (EODT) experiment on wild-type tomatoes.	52
Figure 3.8 RNA quality smear test.	54
Figure 3.9 Block PCR products of DNase treated and synthesized cDNA from all 16 samples together with gDNA of WT (grown under white light) as positive control and water as negative control.	55
Figure 3.10 PCR products using ACTIN primers and PP2Acs primers	56
Figure 3.11: Expression levels of light regulated genes in tomato.	58

List of Tables

Table 2.0: Summary of genotypes used in the PAR and PAR+UV-B experiments.	30
Table 2.1: RNA extracted from plants treated after 4 hours of initial UV-B treatment.	35
Table 2.2 Summary of light treatment experiments	37
Table 3.1: Tomato seeds sowing day and germination percentage	43
Table 3.2: Light regulated genes used in gene expression analysis.	58

Abbreviations

APA	Active binding domain of PHYA
APB	Active binding domain of PHYB
<i>au</i>	<i>aurea</i>
B	Blue light
<i>CHS</i>	Chalcone synthase
COP	CONSTITUTIVE PHOTOMORPHOGENIC 1
CRY	Cryptochrome protein
DET	DEETIOLATED
<i>ein</i>	elongated internode; <i>Brassica phyB</i> mutant
EOD-FR	End-of-day-far-red
EODT-PAR	End-of-day-treatment-PAR
EODT-UV	End-of-day-treatment-UVB
FAD	Flavin adenine dinucleotide
FHL	FAR-RED ELONGATED HYPOCOTYL LIKE
FR	Far-red light
<i>fri</i>	far-red insensitive; tomato <i>phyA</i> mutant
FUS	FUSCA
G	Green light
GA	Gibberellic acid
<i>Ga2ox2</i>	GIBBERELLIC ACID 2 OXIDASES 2
HFR1	HYPOCOTYL IN FAR RED 1
HIR	High irradiance response
HY5	LONG HYPOCOTYL 5
HYH	HY5 HOMOLOG

JA	Jasmonic acid
<i>LeHY5</i>	Tomato LONG HYPOCOTYL 5 gene
LFR	Low fluence response
<i>lh</i>	long hypocotyl
MM	Money maker
nm	Nanometer
PAR	Photosynthetically active radiation
Pfr	Active form of phytochrome capable of absorbing FR light
PHY	Phytochromes
PHYA, PHYB	Phytochrome A, phytochrome B, etc. apoprotein
<i>PHYA, PHYB</i>	Phytochrome A, phytochrome B, etc. gene
phyA, phyB	Phytochrome A, phytochrome B, etc. holoprotein
<i>phyA, phyB</i>	Phytochrome A, phytochrome B, etc. mutant
PIF	PHYTOCHROME INTERACTING FACTOR
Pr	Inactive form of phytochrome capable of absorbing R light
R	Red light
RCC1	Regulator of chromatin condensation 1
SAM	Shoot apical meristem
SAR	Shade avoidance response
<i>sav3-2</i>	mutant with a defect in the TAA1 pathway
FH1	FAR-RED ELONGATED HYPOCOTYL 1
<i>SLIPF</i>	<i>Solanum lycopersicum</i> PHYTOCHROME INTERACTING FACTOR
SPA	SUPPRESSOR OF PHYTOCHROME A
TAA1	Tryptophan aminotransferase of <i>Arabidopsis</i> 1
<i>tri</i>	temporary insensitive; tomato <i>phyB1</i> mutant

Trp	Tryptophan
UV	Ultraviolet light
UVR8	UV-B RESISTANCE LOCUS 8
VLFR	Very low fluence response
WL	White light
WT	Wild-type tomato

