CONTROL OF DEVELOPMENT BY PHYTOCHROME IN THE GARDEN PEA (*Pisum sativum* L.)

by

James L. Weller

B. Sc. (Hons)

Submitted in fulfilment of the requirements

for the degree of

Doctor of Philosophy

Department of Plant Science

University of Tasmania

November 1996

DECLARATION

This thesis does not contain any material which has been submitted for the fulfilment of any other degree or diploma. To the best of my knowledge and belief, this thesis contains no material which has been published, written, or provided by another person, except where due reference is given.

James L Weller

ACKNOWLEDGEMENTS

Thanks to **Jim Reid** for providing seed of mutant lines NEU3 and R83, for arranging my initial visit to the Frontier Laboratory for Photoperception and Signal Transduction at RIKEN in Japan, for financial support during the latter stages of the project, and for allowing me the freedom to pursue my own ideas and directions; to **Ian Murfet** for providing seed of mutant lines Wt10895 and L80m, and for advice and discussion on linkage studies and flowering; to **Ian Cummings, Peter Bobbi, Kathy Mc Pherson,** and **Tracey Jackson** for help with general plant maintenance; to **Leigh Johnson** and **Doug Madden** for construction of the far-red light source used in the Department of Plant Science; to **Rob Warren, Leanne Sherriff,** and **Andrew Poole** for help with scoring, and to **John Ross, Scott Taylor, Christine Beveridge,** and **Steven Swain** for various helpful discussions.

Thanks to **Dick Kendrick** for his generous support and to **Mitsue Ishikawa** and **Hiroko Kobayashi** for assistance during the course of several visits to RIKEN. Thanks also to **Enrique López-Juez** (RIKEN) for assistance with immunoblotting, **Catherine Rameau** (INRA Versailles, France) for selection and provision of the original S2-14 mutant line, and **Akira Nagatani** (RIKEN) for providing anti-pea phytochrome monoclonal antibodies.

Special thanks to **Matthew Terry** for advice about phytochrome extraction and assembly, for permission to include results from assays for phytochromobilin synthesis (Figures 5.7, 5.8, 6.6, 6.7) and heme quantitation (Table 5.3), and for general discussion, support and encouragement during the course of this work. Special thanks also to my parents and to **Garry Deverell, Toby Croft** and **Margot Jones** for extra-curricular support and distraction over the last few years.

ABSTRACT

The phytochrome family is a well-known and important group of biliprotein photoreceptors which mediate effects of light on plant development. This thesis comprises an investigation of the physiological roles of different forms of phytochrome in the garden pea (*Pisum sativum L.*). The presented work focuses on the isolation and subsequent physiological characterization of various phytochrome-deficient mutants of pea, including mutants deficient in phytochrome A, phytochrome B and phytochrome chromophore synthesis. Ethylmethanesulfonate mutagenesis was employed to induce mutation in the pea cultivar Torsdag. M_2 seedlings were screened under red (R) or far-red light (FR) conditions to identify potential phytochrome-deficient mutants. All candidate mutants were back-crossed to the parental line to establish the nature of the genetic control of the mutant phenotypes, and complementation testing was performed between mutant lines showing similar alterations to spectral sensitivity. Four distinct loci were defined by mutants isolated in these screens, corresponding to the previously described *LV* locus and three novel loci designated *FUN1*, *PCD1* and *PCD2*. *LV* and *FUN1* were mapped to linkage groups VI and II, respectively.

Apoprotein-deficient mutants. Mutant *lv* plants are deficient in a phyB apoprotein and show reduced responses to R, while *fun1* mutants are deficient in phytochrome A apoprotein and are unresponsive to FR. Physiological studies suggest that phyB controls seedling phytochrome responses in the LFR mode, whereas phyA controls responses in the VLFR and HIR modes. Double mutants lacking both phyA and phyB reveal (a) that phyA is the only phytochrome controlling responses to continuous FR (b) that both phyA and phyB are required for normal responses to continuous R, and (c) neither phyA nor phyB play a substantial role in inhibition of stem elongation by blue light. Examination of mature plant photoresponses revealed a continuing role for both phyA and phyB in the maintenance of full de-etiolation, and an important role for phyA in photoperiod detection and flower induction.

Chromophore-deficient mutants. Mutant *pcd1* and *pcd2* plants show a reduction in both phyA- and phyB-mediated responses at the seedling stage and a severe depletion of spectrally active phytochrome, consistent with deficiency in the common tetrapyrrole chromophore, phytochromobilin (PΦB). *In vitro* assembly studies, *in vivo* feeding of PΦB precursors, and HPLC-based assay of heme and biliverdin (BV) IXα metabolism in isolated plastids revealed the *pcd1* and *pcd2* mutants to be deficient in the conversion of heme to BV IXα and BV IXα to PΦB, respectively.

Other mutants. In addition to the mutants described above, a dominant mutant (AF05) with strongly enhanced responses to light was also isolated. This mutant has a seedling and flowering phenotype similar to phyA-overexpressing transgenic lines in other species. In addition, the AF05 mutation maps close to *FUN1*, suggesting possible allelism between these loci.

Substantial portions of this thesis have already been published, as follows;

Weller JL, Reid JB (1993) Photoperiodism and photocontrol of stem elongation in two photomorphogenic mutants of *Pisum sativum* L. Planta 189, 15-23

Weller JL, Murfet IC (1994) Location of the *Lv* gene in linkage group VI. Pisum Genet. 26, 41-43

Weller JL, Nagatani A, Kendrick RE, Murfet IC, Reid JB (1995) New *lv* mutants of pea are deficient in phytochrome B. Plant Physiol. 108, 525-532

Weller JL, Terry MJ, Rameau C, Reid JB, Kendrick RE (1996) The phytochrome-deficient *pcd1* mutant of pea is unable to convert heme to biliverdin IXα. Plant Cell 8, 55-67

These papers are included at the end of the thesis, together with other papers published during the course of the work.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviations are defined at first appearance in the text. Standard phytochrome terminology and abbreviations are employed (Quail et al. 1994). For convenience all non-standard abbreviations used are listed below.

aa	amino acid
ALA	5-aminolevulinic acid
B	blue (light)
BV	biliverdin
Chl	chlorophyll
cv.	cultivar
d	day
EMS	ethylmethanesulphonate
EOD	end-of-day
FLR	flower/leaf relativity
FR	far-red (light)
FT	flowering time
G	green (light)
h	hour
HIR	high-irradiance response
IL	white light from incandescent globes
LA	leaflet area
LD	long day(s)
LDP	long-day plant
LE	leaves expanded
LFR	low-fluence response
Lx-y	length between nodes x and y
NFD	node of first open flower
NFI	node of flower initiation
PAL	cv. Paloma
PAR	photosynthetically active radiation
PCB	phycocyanobilin
PFB	phytochromobilin
R	red (light)
RN	reproductive nodes
SD	short day(s)
SDP	short-day plant
SOL	cv. Solara
TBST	Tris-buffered saline/Tween
TN	total nodes
TOR	cv. Torsdag
VLFR	very-low-fluence response
WFL	light from cool white fluorescent tubes
WL	white light
WT	wild type