# Regulatory mechanisms of flowering in Sturt's desert pea (Swainsona formosa)

by

Tanya Tapingkae B Sc Ag (Chiang Mai University, Thailand) M Sc (University of New England, Australia)

A thesis submitted for the degree of Doctor of Philosophy School of Rural Science and Agriculture The University of New England Armidale, N S W Australia

February 2007

#### Acknowledgements

Firstly, I would like to thank my supervisors, Professor Acram Taji and Dr Paul Kristiansen, for their patience, enthusiasm, support and guidance throughout this study. I have learnt so much over the last few years and I would not be here without their guidance, I am most grateful.

I would like to thank Mr Patrick Littlefield of the Electron Microscope Unit for his technical assistance and helpful advice with the scanning electron microscopy study, and I also wish to thank Dr Joelle Coumans-Moens of Molecular and Cellular Biology for her valuable technical help and helpful suggestions on the protein gel electrophoresis study. Appreciation is extended to Mr Kim Quinn for allowing me to use the gel analysis software in the Genetics Research Laboratories.

Many thanks to all the staff and my fellow students in Agronomy and Soil Science, especially Mr Michael Faint, Mr Gary Cluley, Ms Leanne Lisle, Dr Naser Panjehkeh and Dr Masayo Kawaguchi for their help, comments and advice during my time at UNE. In particular I wish to extend an extra thanks to Dr Masayo Kawaguchi for always being there for me.

I wish to thank Dr Jennifer Smith for her help and extremely valuable and constructive criticism on the literature review part of this thesis. Many thanks go to Mr Boyd Gudex for his patience in correcting my English. I am grateful to Associate Professor N. Prakash for helpful comments on the scanning electron microscopy work.

I gratefully acknowledge the financial support provided by the Thai Government and the University of New England Research Assistantship.

Finally, I would like to thank the most important people in my life – my family. Mum, dad and my sisters, thank you for your support, sacrifice and patience during the period of my study in Australia. Thanks to my friends and colleagues for all their support and friendship.

### Statement of originality

I certify that the work presented in this thesis has not been previously submitted for a degree or diploma at any other higher education institution.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another author except where due reference is made.

Tanya Tapingkae

## List of abbreviations and glossary of terms

ABA	abscisic acid
BA	benzyl adenine
BAP	benzylamino purine
С	carbon
[CH <sub>2</sub> O] <sub>n</sub>	carbohydrate
EDTA	ethylenediamine tetra-acetic acid
FW	fresh weight
GA	gibberellin
GA <sub>3</sub>	gibberellic acid
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
LD	long-day
LSD	least significant difference
mRNA	messenger ribonucleic acid
MS	Murashige and Skoog (1962) basal medium
Ν	nitrogen
NAA	$\alpha$ -naphthalene acetic acid
PAGE	polyacrylamide gel electrophoresis
PAR	photosynthetically active radiation (usually $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )
PBZ	paclobutrazol
PGR	plant growth regulator
ppm	parts per million
PPFD	photosynthetic photon flux density ( $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )
rpm	revolutions per minute
RNA	ribonucleic acid
Rubisco	ribulose-1, 5-bisphosphate carboxylase/oxygenase
SD	short-day
SDS	sodium dodecylsulphate

#### Abstract

*Swainsona formosa* (G.Don) J. Thompson (Sturt's desert pea) is an Australia native legume which has potential as an ornamental pot plant and is also suitable for hanging baskets and as a cut flower. Accurate identification of the timing and control of growth stages is critically important in making management decisions for floricultural crops. One of the impediments to the commercialisation of *S. formosa* as a pot plant is its inability to produce flowers under low light conditions.

The apical meristems of *S. formosa* were investigated by stereomicroscopy and scanning electron microscopy to identify flowering time and stages of floral development. Conversion from the vegetative to the reproductive stages began within 40 to 46 days after seed germination for axillary branches and 46 to 52 days for central stems. The order of floral organ initiation within each whorl is unidirectional, except for the petal whorl, which is simultaneous; the flower is organised into five whorls and shows a pentamerous arrangement of sepals and petals, ten stamens in two whorls and a central carpel. This is the first description of the complete floral ontogeny of a member of tribe Galegeae in papilionoid legumes.

The effect of high light intensity ( $800 \pm 50 \ \mu\text{mol m}^{-2}\text{s}^{-1}$ ) and low light intensity ( $150 \pm 10 \ \mu\text{mol m}^{-2}\text{s}^{-1}$ ) on the flowering of *S. formosa* was investigated, with particular emphasis on measuring the changes in sugar concentrations in the transition stages of the shoot apices from the vegetative to the floral, using high-performance liquid chromatography. Plants grown under high light initiated flowers within 45 days from seed germination, while plants grown under low light intensity remained vegetative and produced no flowers during the 60-day experimental period. Trace amounts of glucose (0.52 mg g<sup>-1</sup>) were detected at the beginning of the transition from the vegetative to floral stage (40 days after germination) in the apices of plants grown under high light intensity conditions; this increased to 2.70 mg g<sup>-1</sup> with progressive floral development. No glucose was detected in the shoot apices of plants grown under low light during the experimental period. The timing of changes in sucrose quantity matched the timing of morphological changes, indicating a key role for sucrose in flower development. The changes in the sugar composition of the shoot apices are associated with quantitative

changes in sugar translocation and changes in enzyme activity related to floral transition. Also, the balance between glucose, fructose and sucrose is important for flowering to occur. Adequate amounts of glucose is required for floral initiation and development in *S. formosa*.

Among many factors, the Carbon:Nitrogen (C:N) ratio is considered to be one of the most important physiological signals that induce flowering of *S. formosa*. Reduced %N and %C at the early transition stage, and an increased C:N ratio in the shoot apices, could be used as indicators for stage changes. To flower, plants need to maintain an appropriate C:N ratio. The mode of N acquisition (fixation versus assimilation) seems to influence %N accumulation in the shoot apices of low light grown plants.

To reach a more precise conclusion on the effects of carbohydrates on flowering of S. formosa, the effects of exogenous sugar applications on the flowering of S. formosa were tested. Several experiments (spraying, brushing, injecting and absorbent cotton on apex), on the application of exogenous sugars, were conducted to identify the correct methodology of application, and the types and concentrations of sugars needed to induce flowering of S. formosa grown under low light intensity conditions. Results confirm that S. formosa requires adequate high light intensity for flower induction and development. All the sugar treatments using exogenous application methods failed to induce the transition to flowering in S. formosa plants grown under low light conditions. The failure to flower depends on many factors, such as the use of ineffective concentrations of sugars along with inappropriate application methods. In vitro flowering was achieved within 4 to 6 weeks by placing shoot apices from high light grown stock plants on Murashige and Skoog medium supplemented with 3.0 to 4.5% sucrose. This could be the result of an interplay of factors, including environmental conditions under which the explant source was grown and the types and concentrations of exogenous sugar used in the culture medium. This is the first report of S. formosa producing flowers in vitro. It is suggested that future work on exogenous application of sugars be expanded by using *in vitro* techniques.

The key stages in the transition of *S. formosa* plants to flowering is correlated with protein concentrations and patterns. High light intensity caused changes in the concentrations and types of proteins that promoted flower initiation and differentiation.

These protein changes were absent from the vegetative apices of high light plants and all the apices of plants grown under low light. Further studies on protein morphogenetic markers, as well as their genes and conditions for expression, are among the most promising approaches to the regulation of morphogenetic processes in this and other plants.

## Table of contents

Statement of originalityIIIList of abbreviations and glossary termsIVAbstractVTable of contentsVIIIList of tablesXIIIList of figuresXIVChapter 1 General Introduction11.1 Introduction11.2 Research aims31.3 Thesis outline3Chapter 2 Literature Review42.1 Introduction42.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6.1 Models of floral development172.6.1 Models of floral development ontrol182.6.2 Floral development172.6.1 Models of floral development172.6.2 Floral development172.6.2 Floral development172.6.2 Floral development172.6.2 Floral development172.6.2 Floral development182.6.2 Floral development182.6.2 Floral development182.6.2 Floral development172.6.2 Floral development182.6.2 Floral development17	Acknowledgements	II
List of abbreviations and glossary termsIVAbstractVTable of contentsVIIIList of tablesXIIIList of figuresXIVChapter 1 General Introduction11.1 Introduction11.2 Research aims31.3 Thesis outline3Chapter 2 Literature Review42.1 Introduction42.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Development atages in the life cycle of flowering plants132.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	Statement of originality	III
Abstract       V         Table of contents       VIII         List of tables       XIII         List of figures       XIV         Chapter 1 General Introduction       1         1.1 Introduction       1         1.2 Research aims       3         1.3 Thesis outline       3         Chapter 2 Literature Review       4         2.1 Introduction       4         2.2 Swainsona formosa       4         2.2.1 The flower       6         2.2.2 Flower development       7         2.3 General cultivation       9         2.4 Factors affecting growth and development of <i>S. formosa</i> 10         2.4.1 Environmental conditions       10         2.4.2 Plant growth regulators       11         2.4.3 Pests and diseases       12         2.5 Developmental stages in the life cycle of flowering plants       13         2.5.1 The flowering process       14         2.5.1.2 Floral evocation or initiation       16         2.5.1.3 Floral development       17         2.6.1 Models of floral development       17         2.6.1 Models of floral development control       18         2.6.2 Floral development in pea ( <i>Pisum sativum</i> )       20	List of abbreviations and glossary terms	IV
Table of contents       VIII         List of tables       XIII         List of figures       XIV         Chapter 1 General Introduction       1         1.1 Introduction       1         1.2 Research aims       3         1.3 Thesis outline       3         Chapter 2 Literature Review       4         2.1 Introduction       4         2.2 Swainsona formosa       4         2.2.1 The flower       6         2.2.2 Flower development       7         2.3 General cultivation       9         2.4 Factors affecting growth and development of <i>S. formosa</i> 10         2.4.1 Environmental conditions       10         2.4.2 Plant growth regulators       11         2.4.3 Pests and diseases       12         2.5 Developmental stages in the life cycle of flowering plants       13         2.5.1.1 Floral induction       14         2.5.1.2 Floral evocation or initiation       16         2.5.1.3 Floral development       17         2.6.1 Models of floral development control       18         2.6.2 Floral development in pea ( <i>Pisum sativum</i> )       20	Abstract	V
List of tables       XIII         List of figures       XIV         Chapter 1 General Introduction       1         1.1 Introduction       1         1.2 Research aims       3         1.3 Thesis outline       3         Chapter 2 Literature Review       4         2.1 Introduction       4         2.2 Swainsona formosa       4         2.2.1 The flower       6         2.2.2 Flower development       7         2.3 General cultivation       9         2.4 Factors affecting growth and development of <i>S. formosa</i> 10         2.4.1 Environmental conditions       10         2.4.2 Plant growth regulators       11         2.4.3 Pests and diseases       12         2.5 Developmental stages in the life cycle of flowering plants       13         2.5.1.1 Floral induction       14         2.5.1.2 Floral evocation or initiation       16         2.5.1.3 Floral development       16         2.6 The genetic basis of flower development control       18         2.6.2 Floral development in pea ( <i>Pisum sativum</i> )       20	Table of contents	VIII
List of figuresXIVChapter 1 General Introduction11.1 Introduction11.2 Research aims31.3 Thesis outline3Chapter 2 Literature Review42.1 Introduction42.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	List of tables	XIII
Chapter 1 General Introduction11.1 Introduction11.2 Research aims31.3 Thesis outline31.3 Thesis outline3Chapter 2 Literature Review42.1 Introduction42.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	List of figures	XIV
1.1 Introduction11.2 Research aims31.3 Thesis outline31.3 Thesis outline3Chapter 2 Literature Review42.1 Introduction42.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6.1 Models of flower development control182.6.2 Floral development in pea ( <i>Pisum satiyuun</i> )20	Chapter 1 General Introduction	1
1.2 Research aims31.3 Thesis outline3Chapter 2 Literature Review42.1 Introduction42.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6.1 Models of flower development on trol182.6.2 Floral development in pea ( <i>Pisum satiyum</i> )20	1.1 Introduction	1
1.3 Thesis outline       3         Chapter 2 Literature Review       4         2.1 Introduction       4         2.2 Swainsona formosa       4         2.2 Swainsona formosa       4         2.2.1 The flower       6         2.2.2 Flower development       7         2.3 General cultivation       9         2.4 Factors affecting growth and development of <i>S. formosa</i> 10         2.4.1 Environmental conditions       10         2.4.2 Plant growth regulators       11         2.4.3 Pests and diseases       12         2.5 Developmental stages in the life cycle of flowering plants       13         2.5.1 The flowering process       14         2.5.1.2 Floral evocation or initiation       16         2.5.1.3 Floral development       17         2.6 The genetic basis of flower development       17         2.6.1 Models of floral development control       18         2.6.2 Floral development in pea ( <i>Pisum sativum</i> )       20	1.2 Research aims	3
Chapter 2 Literature Review42.1 Introduction42.2 Swainsona formosa42.2.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral induction142.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	1.3 Thesis outline	3
2.1 Introduction42.2 Swainsona formosa42.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral induction142.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	Chapter 2 Literature Review	4
2.2 Swainsona formosa42.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.1 Introduction	4
2.2.1 The flower62.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development172.6 The genetic basis of flower development172.6.1 Models of floral development in pea ( <i>Pisum sativum</i> )20	2.2 Swainsona formosa	4
2.2.2 Flower development72.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral induction142.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.2.1 The flower	6
2.3 General cultivation92.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.2.2 Flower development	7
2.4 Factors affecting growth and development of <i>S. formosa</i> 102.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.3 General cultivation	9
2.4.1 Environmental conditions102.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.4 Factors affecting growth and development of S. formosa	10
2.4.2 Plant growth regulators112.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5 Developmental stages in the life cycle of flowering plants142.5.1 The flowering process142.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.4.1 Environmental conditions	10
2.4.3 Pests and diseases122.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.4.2 Plant growth regulators	11
2.5 Developmental stages in the life cycle of flowering plants132.5.1 The flowering process142.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.4.3 Pests and diseases	12
2.5.1 The flowering process142.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.5 Developmental stages in the life cycle of flowering plants	13
2.5.1.1 Floral induction142.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.5.1 The flowering process	14
2.5.1.2 Floral evocation or initiation162.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.5.1.1 Floral induction	14
2.5.1.3 Floral development162.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.5.1.2 Floral evocation or initiation	16
2.6 The genetic basis of flower development172.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.5.1.3 Floral development	16
2.6.1 Models of floral development control182.6.2 Floral development in pea ( <i>Pisum sativum</i> )20	2.6 The genetic basis of flower development	17
2.6.2 Floral development in pea ( <i>Pisum sativum</i> ) 20	2.6.1 Models of floral development control	18
	2.6.2 Floral development in pea ( <i>Pisum sativum</i> )	20

2.7 The control of flowering	23
2.7.1 Internal factors	23
2.7.1.1 Plant maturity	23
2.7.1.2 Nutrition	24
Carbohydrates	24
Carbon:Nitrogen (C:N) ratio	26
Carbon and Nitrogen metabolisms	28
2.7.1.3 Protein	29
2.7.1.4 Plant growth regulators and other compounds	31
2.7.2 External or environmental factors	36
2.7.2.1 Light	36
Light intensity	37
Light spectrum	38
Light duration or photoperiod	39
2.7.2.2 Temperature	40
2.7.2.3 Stress	41
2.8 In vitro flowering	43
2.8.1 Carbohydrates	43
2.8.2 Mineral nutrients	44
2.8.3 Phytohormones	44
2.9 Chapter summary	45
Chapter 3 General Materials and Methods	47
3.1 Seed germination	47
3.2 Seedling establishment	47
3.3 Statistical analysis	50
Chapter 4 Conversion from Vegetative Stage to Reproductive Sta	ge and Floral
Ontogeny of Swainsona formosa	51

4.1 Introduction	51
4.2 Materials and methods	52
4.2.1 Plant materials	52
4.2.2 Scanning electron microscopy	53
4.3 Results	53

4.3.1 The time of transformation from the vegetative stage to	
the reproductive stage	53
4.3.2 Morphology and ontogeny of the S. formosa flower	
and inflorescence	54
4.4 Discussion	60
4.4.1 The time of transformation from the vegetative stage	
to the reproductive stage	60
4.4.2 Morphology and ontogeny of the S. formosa flower	
and inflorescence	64
4.5 Conclusion	66

01
66
noot Apices during
68
68
69
69
70
70
70
73
79

Chapter 6 The Relationships of Carbon and Nitrogen in the Shoot Apices of	' Swainsona
formosa at Different Stages of Development	80
6.1 Introduction	80
6.2 Materials and methods	81
6.2.1 Plant material and growth conditions	81
6.2.2 Carbon:Nitrogen ratio analysis	82
6.2.3 Statistical analysis	82
6.3 Results	82
6.4 Discussion	85
6.5 Conclusion	87

Chapter 7 Effect of Exogenous Sugar Application on the Flowering of Swainsona formosa 89

7.1 Introduction 89

7.2 Materials and methods	90
7.2 Matchais and methods	70

- 7.2.1 *In vivo* (Experiment 1, 2, 3 and 4) 90
  - 7.2.1.1 Experiment 1: Effect on flowering of types and concentrations and frequency of application of sugar spray applied to *S. formosa* plants grown under low light intensity conditions. 90
  - 7.2.1.2 Experiment 2: Effect on flowering of types and concentrations and frequency of application of sugar applied with a brush to the apical bud of *S. formosa* plants grown under low light intensity conditions.
  - 7.2.1.3 Experiment 3: Effect on flowering of types and concentrations of sugar applied by placing a piece of absorbent cotton onto the apex of *S. formosa* plants grown under low light intensity conditions.
  - 7.2.1.4 Experiment 4: Effect on flowering of types and concentrations of sugar injected into apices of *S. formosa* plants grown under low light intensity conditions.

7.2.2 In vitro	(Experiment 5)	92
······································		

7.2.2.1 Experiment 5: Influence of carbohydrate source and concentration on the *in vitro* flowering of shoot culture of *S. formosa.*7.2.3 Data collection and statistical analysis
7.2.3.1 *In vivo* (Experiment 1, 2, 3 and 4)
7.2.3.2 *In vitro* (Experiment 5)
95

7.3 Results	95
7.4 Discussion	96
7.5 Conclusion	101

Chapter 8 Comparison of Protein Expression in the Shoot Apices at the Vegetative andFlowering Stages in Swainsona formosa1038.1 Introduction1038.2 Materials and methods104

8.2.1 Plant material and growth conditions	104
8.2.2 Protein extraction	105
8.2.3 Protein quantification	105
8.2.4 One-dimensional gel electrophoresis	106
8.2.5 Protein staining	106
8.2.6 Gel analysis	106
8.3 Results	107
8.4 Discussion	110
8.4.1 Protein concentrations and composition: differences in	vegetative and
reproductive apices	111
8.4.2 Protein concentrations and composition: variation in repr	oductive apices
over time	112
8.5 Conclusion	113
Chapter 9 General Discussion and Future Directions	114
9.1 Introduction	114
9.2 Summary and consolidation of the literature review	114
9.3 Floral ontogeny of Swainsona formosa	115
9.4 Changes in sugar levels, Carbon:Nitrogen ratio and protein ex	pression in the
shoot apices during the transition period from the vegetative to	the floral stage
	116
9.5 Effect of exogenous sugar applications on flowering	117
9.6 Directions for future research	118
References	120
Appendix	150

### List of tables

<b>Table 2.1</b> Effects of some plant growth regulators (PGRs) on Swainsona formosa.	12
<b>Table 2.2</b> Critical genes in flower development in pea ( <i>Pisum sativum</i> ).	22
<b>Table 2.3</b> Effects on flowering in some plant species of plant growth regulators related compounds that function as hormones.	and 32
Table 2.4 Effects on flowering in some plant species of some plant growth retarda	nts. 35
<b>Table 4.1</b> Developmental stages of Swainsona formosa in relation to time after s         germination.	eed 54
<b>Table 4.2</b> Sequence of stages in the flowering process of Swainsona formosa.	55
<b>Table 5.1</b> Shoot apex development of <i>Swainsona formosa</i> plants grown under high l conditions (800 $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> ).	ight 69
Table 6.1       Sampling development stages (Days after seed germination)         Carbon:Nitrogen ratio analysis of the shoot apices of Swainsona form         plants grown under high or low light intensity.	for 2 <i>05a</i> 82
<b>Table 7.1</b> Chemical constituents for the preparation of 1 L of MS basal medium.	92

**Table 8.1** Sampling development stages (Days after seed germination) for proteinprofiling of the shoot apices of Swainsona formosa grown under high lightintensity and low light intensity.105

#### List of figures

- Figure 2.1 Distribution of Swainsona formosa in Australia from 439 recorded mappedsites (Australia's Virtual Herbarium, 2003).5
- Figure 2.2 (A) Flower of Swainsona formosa. (B) Pods and seeds of Swainsona formosa.
   6

Figure 2.3 The seven stages of flower development of *Swainsona formosa*. 8

**Figure 2.4** The four major genetic pathways regulating flowering time in *Arabidopsis*. AP1 = APETALA1, CO = CONSTANTS, FCA = FLOWERING CA, FVE = FLOWERING VE, FLC = FLOWERING LOCUS C, FT = FLOWERING LOCUS T, LFY = LEAFY, SOC1 = SUPPRESSOR OF OVEREXPRESSION OF CO1, VRN1 = VERNALISATION1, VRN2 = VERNALISATION2. 18

Figure 2.5 Classical ABC model.

Figure 2.6 Floral ontogeny of *Arabidopsis* and *Antirrhinum* flowers (left) compared to *Pisum* and *Medicago* (right). F = Floral meristem; S = sepal primordium; CP common primordium; P = petal primordium; St = stamen primordium; C = carpel primordium.

Figure 2.7 The synthesis and hydrolysis of sucrose (disaccharide).25

Figure 2.8 Simplified overview of primary C and N metabolism in photosynthetic cells of higher plants. Enzyme name are given in boxes, PK = pyruvate kinase, AGPase = ADP glucose phosphorylase, CS = citrate synthase, ICDH = isocitrate dehydrogenase, RuBP = ribulose-1,5-bisphosphate, 3PGA = 3-phosphogycerate, PEP = phosphoenolpyruvate, Pyr = pyruvate, IC = isocitrate, Glu1P = glucose-1-phosphate.

Figure 3.1 Swainsona formosa plants growing in the growth cabinet.48

19

- Figure 3.2 Swainsona formosa stems were staked using bamboo rods to support upright growth growing.
   49
- **Figure 4.1** Stage 0: (A) vegetative apex showing one leaf primordium, with its paired stipules on either side (< 40 days after seed germination). (B) Showing two leaf primordia (the younger with two stipule primordia). st = stipule primordium; lp = leaf primordium. Scale bar =  $100 \mu m$ . 57
- **Figure 4.2** Stage 1: (*A*) early transition stage (~ 40 45 days after seed germination) showing a floral primordium in the axil of a leaf primordium. (*B*) General view of apex in which the floral primordia can be distinguished. st = stipule primordium; lp = leaf primordium; fp = floral primordium. Scale bar *A* = 50  $\mu$ m; *B* = 100  $\mu$ m. 57
- Figure 4.3 Stage 2: (A) inflorescence meristem showing five floral buds (~ 45 50 days after seed germination). (B) General view at the floral initiation stage.
  (C) Lateral view of (B). (D) Top view showing five floral buds. Br = bract;
  F = floral bud. Scale bar = 100 μm.
- **Figure 4.4** Reproductive meristem showing continued development of individual floral buds. Bracts removed. (*A*) Floral apex prior to bracteole initiation, showing a bare floral apex in the axil of a bract or leaf, before any organs form on it. (*B*) Showing a floral apex after two bracteoles form on the two sides. (*C*) Stage 3: the first sepal primordium visible ( $\sim 50 - 55$  days after seed germination). (*D*) Showing completion of sepal initiation. (*E*) Continued development of abaxial sepal primordium and two lateral sepal primordia. (*F*) All sepals visible. (*G*) Sepals cover the rest of the floral primordia. (*H*) Lateral view of (*G*). Br = bracteole; Sab = abaxial sepal primordium; SI = lateral sepal primordia; S = sepal. Scale bar  $A = 20 \ \mu m$ ;  $B-E = 50 \ \mu m$ ; F-H =100  $\mu m$ .
- Figure 4.5 Stage 4: (A) a carpal primordium and all five petal primordia are initiated ( $\sim$  50 55 days after seed germination). (B) Appearance of first antesepalous

stamen between the two petal primordia at the bottom of the micrograph. Bracts were removed. C = carpel primordium; P = petal primordium; S = sepal; A = antesepalous stamen. Scale bar  $A = 100 \ \mu m$ ;  $B = 50 \ \mu m$ . 59

- **Figure 4.6** Stage 5: Sepals removed. (*A*) all five antesepalous or outer stamen primordia, alternate with petal primordia (~ 55 – 60 days after seed germination). (*B*) Showing all five antesepalous primordia at a slightly later stage. (*C*) Well-formed antepetalous or inner stamen primordial. (*D*) Completely differentiated floral primordia. (*E*) Carpel with cleft which will form the locule of the ovary. (*F*) Stamens elongate and begin to differentiate anthers and filaments. (*A-D*) apical view of single flower bud. (*E-F*) lateral view of single flower bud. P = petal; C = carpel; S = sepal; A = antesepalous stamen (outer); a = antepetalous stamen (inner). Scale bar *A-B* = 50 µm.; *C-H* = 100 µm. 59
- Figure 4.7 Stage 6: (A) all floral parts are present and identifiable (about 60 70 days after seed germination). (B) Anthers and pistil fully differentiated. All sepals were removed to facilitate observation. P = petal; pt = pistil; at = anther. Scale bar  $A = 100 \mu m$ ; B = 1 mm.
- Figure 4.8 Stage 7: (A) showing individual flower. (B) Inflorescence (umbel) showingfive to eight flowers (about 80 days after seed germination).60
- Figure 4.9 Diagram showing proportional overlap among whorls of floral organs inPisum sativum and Swainsona formosa.66
- Figure 5.1 Changes in sugar content (sucrose, glucose and fructose) in the shoot apices of *Swainsona formosa* plants grown under high light intensity (H, 800  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>) and low light intensity (L, 150  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>) during the study period of 60 days. Results are the means  $\pm$  standard error from at least four repetitions. 72
- **Figure 5.2** Changes in sugar content (sucrose, glucose and fructose) in the shoot apices of *Swainsona formosa* plants grown under high light intensity (800 μmol m<sup>-</sup>

 $^{2}s^{-1}$ ) during the study period of 80 days. Results are the means  $\pm$  standard error from at least four repetitions. 72

- Figure 5.3 Changes in sucrose: glucose ratio in the shoot apices of *Swainsona formosa* plants grown under high light intensity (800 μmol m<sup>-2</sup>s<sup>-1</sup>) during the first 60 days of the study period.
- **Figure 6.1** Relative concentrations of carbon:nitrogen (C:N) ratio in shoot apices of *Swainsona formosa* on a dry weight basis. For the treatments, High = high light intensity (800  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>) and Low = low light intensity (150  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>). Results are the means  $\pm$  standard error of four repetitions. 84
- Figure 6.2 Percentage of carbon in shoot apices of *Swainsona formosa* on a dry weight basis. For the treatments, High = high light intensity (800  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>) and Low = low light intensity (150  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>). Results are the means  $\pm$ standard error of four repetitions. 84
- **Figure 6.3** Percentage of nitrogen in shoot apices of *Swainsona formosa* on a dry weight basis. For the treatments, High = high light intensity (800  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>) and Low = low light intensity (150  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>). Results are the means  $\pm$  standard error of four repetitions. 84
- Figure 7.1 Application methods for applying the exogenous sugar to the Swainsona formosa plants (A) spraying method (B) brush painting method (C) placing a piece of absorbent cotton onto the apex method (D) injection method (E) in vitro method.
- Figure 7.2 Leaves of plants showing thrips infestation at high sugar concentration (100  $g L^{-1}$ ) applied as foliar spray. Scale bar = 1 cm. 95
- Figure 7.3 In vitro flowering of Swainsona formosa (A) In vitro flowering on MS medium supplemented with 4.5% sucrose. (B) Inflorescence just after emergence from shoot. Scale bar = 1 cm.

- Figure 8.1 The changes in protein concentrations in *Swinsona formosa* shoot apices for different sampling days (Days after seed germination). Vertical bars represent standard error (n = 3). If no bar is visible, standard error is smaller than symbol size. For the treatments, High = high light intensity and Low = low light intensity. 107
- **Figure 8.2** The electrophoretic separation on acrylamide gels of the soluble proteins of *Swainsona formosa* shoot apices: Changes with time in the protein patterns. std = molecular weight of marker given in kiloDaltons (kDa). 60L = apices grown in low light intensity for 60 days. Gel lanes were loaded with equal amounts of protein (100 µg).
- Figure 8.3 The presence and/or absence of the 34, 51, 66, 79 and 89 kDa bands over time in *Swainsona formosa* shoot apices. 60L = apices grown in low light intensity for 60 days. Gel lanes were loaded with equal amounts of protein (100 µg).