The molecular basis for the initiation of fruit development and parthenocarpy

by

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A thesis submitted for the degree of

Doctor of Philosophy

at

The University of Adelaide

Department of Plant Science

in collaboration with

CSIRO Plant Industry, Horticulture Unit

Urrbrae, Adelaide

1st November, 2000

This thesis is submitted on archival paper

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Abstract

Parthenocarpy, or seedless fruit development, has an agronomic importance in many horticultural crops. In most fruit, fertilization or seed set usually determines whether fruit growth is sustained. Naturally occurring parthenocarpy results from a genetic lesion that permits fruit to develop in the absence of fertilization and seed development. Parthenocarpy can also be induced artificially with cytokinin, gibberellin or auxin plant growth regulators applied to anthesis pistils. This thesis describes genetic research using *Arabidopsis* as a model plant to identify integral mechanisms that control parthenocarpy and the initiation of fruit development.

The growth and structure of the *Arabidopsis* pistil was determined post-fertilization. Experiments were designed to understand how plant growth regulators induce *Arabidopsis* silique (fruit) development in emasculated anthesis stage pistils. Exogenous gibberellin (GA_3) induced growth and cellular differentiation most comparable to pollinated pistils. Dependencies on gibberellins during silique development were examined in mutants defective for gibberellin biosynthesis (*ga1*, *ga4-1*, *ga5-1*) or perception (*spy-4*, *gai-1*). Although exogenous GAs are effective at inducing parthenocarpy, mutant studies concluded that GAs are not the sole cue for fruit development in *Arabidopsis*. Mutants blocked in GA perception could develop siliques in response to pollination, auxin, cytokinin but not to exogenously applied gibberellins. Silique structure in pollinated *gai-1* and *ga5-1* provided strong evidence for a model supporting evidence of an auxin-like signal regulating structural development and that GAs limit anticlinal cellular division. A specialized function for *GAI* and related *GRAS* family members in controlling cellular division during fruit development was uncovered.

A mutant that forms parthenocarpic siliques without fertilization (*fwf*), was also characterized. The presence of surrounding floral whorls reduced the extent of parthenocarpic silique formation in *fwf*. Silique growth in the *fwf* background was examined when hormone perception, ovule and carpel identity functions were removed genetically. This established that *FWF* functions independent of *GAI*-mediated GA perception. Carpel identity conferred by *FUL* was critical for parthenocarpic silique elongation and ovule development beyond integument initiation, nucellar specification and subsequent morphogenesis, was essential for parthenocarpic silique development in *fwf*. Silique elongation occurs over a four-day period post-pollination or post-anthesis. This coincides with a similar time period in which *fwf* ovules remained receptive to fertilization. These observations are congruent with the hypothesis that *FWF* potentially represses a signal transduction process initiated within the ovule that mediates subsequent transition from carpel to silique development. Further analysis revealed that *aberrant testa shape* (*ats*) a mutant defective in integument formation enhanced parthenocarpic development in *fwf*, indicating that an ovule located repressor other than *fwf* can function to affect silique formation.

Other studies have shown that ethylene can modulate auxin-dependent growth in both aerial and root tissues by altering both polar and lateral auxin transport. The contribution of ethylene perception to signal transduction between ovule and carpel was also genetically assessed. Constitutive ethylene responses, conferred by *ctr1-1*, enhanced cellular expansion in *fwf* and also the autonomous silique development in *fis-2,* which develops autonomous endosperm. *ats ctr1-1* and *ino ctr1-1* double mutants were also found to be parthenocarpic. This indicates that ethylene perception and integumentary structure play an important role in autonomous silique development, conceivably by

changing the polar and lateral movement of an auxin-like signal within the integumentary tissues of the ovule.

fwf and *ats* were fine mapped on chromosome 5 of *Arabidopsis*. Candidate genes were identified corresponding to both mutations but only the identity of *FWF* was established. *Auxin Response Factor 8* (*ARF8*) was cloned and sequenced from the *fwf* mutant background. The gene encodes a protein with a amino-terminal DNA binding domain and a carboxy-terminal protein binding domain which homo- and hetero-dimerizes with other ARF or Aux / IAA class proteins. *ARF8* sequence from *fwf* mutants encoded a mutation in the translation start site. Complementation of *fwf* plants by the transformation of wild type copies of *ARF8* into *fwf* plants was hampered by reduced transformation efficiency. However wild type L.*er* and No.O plants transformed with mutant copies of *ARF8* were obtained in higher frequency, and these formed parthenocarpic siliques when primary transformants were emasculated. This indicated that an interfering protein is produced from the mutated *ARF8* gene that has altered regulatory activity. Sequence analysis indicated this and found that interference resulted from functional activity of the Q-rich and carboxy-terminal domains of the ARF8 protein. This inference is consistent with other published molecular data, which has demonstrated that the carboxy-terminal domain, together with the Q-rich region of selected ARF members, can activate auxin-responses. Thus the FWF / ARF8 protein may have a dual role, repressing carpel growth development through the DNA binding domain and then ensuring activation of silique development through the carboxy-terminal domain.

The combined molecular and genetic data has been used to construct models concerning the genetic control of silique development. The first model considers the role of plant hormones and how signals from floral whorls surrounding the carpel and from within the ovule control silique growth. A model is also presented for the control of adaxial

growth and development of the outer integument by the *INNER NO OUTER* gene. Finally the role of *FWF* and *SPY* in controlling floral tissue identity and boundary tissue specification is considered in a third model. Modification of the *FWF* / *ARF8* gene could be used as a tool to improve fruit set and retention in horticultural crops, in addition to creating seedless parthenocarpic fruit.

Declaration

I declare that this work contains no material, which has been accepted for the award of any other degree or diploma in any University or any other tertiary institution. To the best of my knowledge and belief this thesis is original and contains no material previously written or published by another person, except where due reference has been made in the text.

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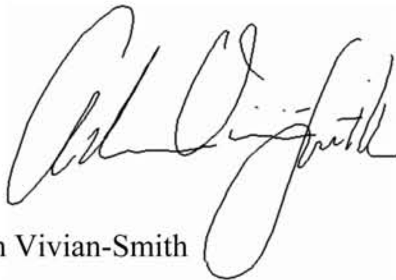

Adam Vivian-Smith

November, 2000

Acknowledgements

Firstly I would like to thank my supervisor Dr Anna Koltunow for her patience, encouragement and also for providing me with the opportunity to work in her lab on such a great project. This project and her support have provided me with many stimulating years of enjoyment.

I would like to thank Luo Ming for isolating the *fwf* mutant and for generous technical advice, Carol Horsman and Dr Anna Koltunow for their excellent illustration skills in Figure 3.1, and also to Carol Horsman for DNA preparation, help with transformation of *ARF8* clones and looking after plants. I thank Dr Anna Koltunow for performing cloning steps involved in the *ARF8* complementation, and I thank the Australian Genome Research Facility for sequencing *ARF8*.

I also would like to thank Prof Peter Langridge, Dr Susan Barker, and Dr Abed Chaudhury for helping to make this project possible and also for their advice during the project. I also thank the University of Adelaide for the Australian Postgraduate Award and the Horticultural Research and Development Corporation and the Commonwealth Scientific Industrial Research Organization (Australia) for funding and supplementary scholarship. I also thank the organizing members of the 10th International Arabidopsis conference for the travel scholarship to the 11th Arabidopsis conference in Madison, Wisconsin, 24th to 28th June 2000.

I thank Dr Kay Schneitz for providing *ant* and *ino* alleles, Dr Steve Swain for *spy-4*, Jason Walker and Prof John Larkins for molecular markers, and the *Arabidopsis* Biological Resource Center for seed. Dr Lyn Waterhouse at CEMMSA provided help with SEM microscopy facilities.

Special thanks go to past and present lab members, Susan, Nick, Matt Tucker, Matt Lynch, Sandra, Georgina, Jenny, Yoshi, Tohru, Debbie, Peter and Anne, for their technical know-how, payouts, chats and their discussions. I thank Dr Cameron McConchie and Dr Anna Williamson for inspiring me to pursue a career in science.

Warmest thanks go to Clare Gambley, Bec Short, Jade Herriman, Penny Richards, Luke Faulkner, Bree Bickmore, Kirrilley Becker, Toby Bramwell, Bart Rossel, Flavia Pellerone, Toby Knight, Anthony Otten, Danielle Smyth, Anna Stines, Jacqui Martin, Kylie Noonan, Dave Tattersall, Sarah and Phil Bricher, Steph Vaughan, Monaz Mehta, Abbie Collinson, Ava Blässe, the Reservoir Dogs and Whatthefoxhat? football teams and all other friends for enduring support, riotous behavior and memorable times.

Lastly, tremendous thanks go out to Ashley Bowen, Gabrielle, Brendan, Blake and my parents for never-ending support, wisdom and encouragement, right from the start of my study days. This thesis is dedicated to you.

Abbreviations

A	adenine
AFLP	Amplified fragment length polymorphism
ARF	auxin response factor
BA	benzyl adenine
bp, kbp, mbp	nucleotide base pairs, kilobase pairs, megabase pairs
CAPS	cleaved amplified polymorphic sequence
C	cytosine
cDNA	deoxyribonucleic acid complementary to mRNA
cm, mm, μm	centimetre, millimetre, micrometre
Col	*Arabidopsis* Columbia ecotype
dH_20	distilled water
DNA	deoxyribonucleic acid
DPA	days post-anthesis
EDTA	ethylenediaminetetraacetic acid
er	*erecta* mutation
G	guanine
GA, GA_X	gibberellin, gibberellic acid X,
g, mg, µg	gram(s), milligram(s), microgram(s)
IAA	indole acetic acid
L.*er*, L.*ER*	*Arabidopsis* Landsberg *erecta*, *Arabidopsis* Landsberg *ERECTA* ecotypes

L, ml, μl	litre(s), millilitre(s), microlitre(s)
M	molarity
min, hr	minute(s), hour(s)
mol	moles
mRNA	messenger ribonucleic acid
n	number of replicate measurements
NAA	napthyl acetic acid
°C	degrees celsius
PGR	plant growth regulator
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	Sodium dodecyl sulphate
SSLP	simple sequence length polymorphism
T	thymidine
TAE	Tris-acetate-EDTA
Taq	*Thermus aquaticus* DNA polymerase
TE	Tris-EDTA buffer
Tris	Tris[hydroxymethyl]amino methane
U	Units of enzyme
%(v/v)	percent volume per volume
%(w/v)	percent weight volume