Molecular & cytological aspects of
seed development in sexual &
apomictic *Hieracium*

by
Matthew Robert Tucker
B. Biotech (Hons)

A thesis submitted for the degree of

Doctor of Philosophy

at

The University of Adelaide,
Department of Agricultural Science

in collaboration with

CSIRO Plant Industry,
Horticulture Unit

Urrbrae, Adelaide
April 26th 2003
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Abstract

Sexual reproduction in angiosperms is a highly regulated process that begins with the formation of a flower and ends with the formation of seeds. The ovule is the progenitor of the seed and during the course of reproduction it is the site of embryo sac formation, double fertilisation and embryo and endosperm development. Asexual seed reproduction in Hieracium, referred to as apomixis, is characterised by the formation of an embryo sac(s) without meiosis, and an embryo and endosperm without fertilisation. The molecular processes controlling apomixis are unknown.

In this study, molecular and cytological aspects of fertilisation-independent (autonomous) endosperm development were investigated in Hieracium. Early autonomous endosperm divisions were irregular in the apospatic when compared to fertilisation-dependent endosperm divisions in the sexual plant. However, the general morphology of dividing syncytial nuclei and endosperm cells, and the expression patterns of the A-MEA:GUS, A-FIS2:GUS and A-FIE:GUS chimeric genes were strikingly similar in sexual and apospatic plants throughout endosperm development. Flow cytometry analyses showed that seeds arising from apomictic Hieracium displayed a higher level of heterogeneity compared to those from the sexual plant. These findings emphasised the presence of overlaps between sexual and apospatic processes in facultative apomictic Hieracium.

Hypotheses suggest that in apomictic plants, altered expression or function of the FERTILISATION INDEPENDENT SEED (FIS) genes, MEA, FIS2 and FIE, which regulate endosperm initiation in sexual plants, may result in the formation of autonomous endosperm. A full-length FIE homologue was identified from sexual
and apomictic *Hieracium* plants, and function of the apomict equivalent of *FIE* was verified by genetic complementation of the *Arabidopsis fie-2* mutant. Further genomic characterisation and RNAi silencing studies suggested that the *HFIE* gene from apomictic *Hieracium* was required for viable autonomous seed development. Mutations in *HFIE* are unlikely to be the cause of autonomous endosperm development in apomictic *Hieracium*.

The cytological and molecular data obtained from this thesis provide evidence that apomictic and sexual pathways share common regulatory elements to produce a seed. The findings support models that suggest apomixis is caused by mutations in a key regulatory sexual gene(s) or by changes in gene expression induced by epigenetic factors.¹

¹ Findings from this thesis were combined with data from other *Hieracium* developmental marker studies in a paper published in *The Plant Cell*.