Epigenetic regulation and inheritance of autonomous seed development in apomictic *Hieracium*

Julio Carlyle M Rodrigues

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School of Agricultural Food and Wine

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Abstract

Apomixis is an intriguing and agronomically valuable asexual reproductive pathway resulting in seeds that give rise to plants that are identical in genotype to the female parent. Apomixis is absent in agriculturally important seed crops and our work has focused on the analysis of apomixis in the daisy-like genus *Hieracium* which contains sexual and apomictic species. Prior studies have shown that apomixis in *Hieracium* is controlled by two dominant loci. The LOA controls the avoidance of meiosis during female gametophyte (embryo sac) formation and the LOP locus is required for fertilization independent embryo formation during seed initiation. The genes conferring apomixis are unknown. In this study we focused on the events of autonomous seed initiation.

Cytological examination of apomictic mutants that have lost LOA or LOP and analysis of their progeny enabled us to characterize developmental aspects associated with the function of these loci. Upon removal of LOA meiosis occurs normally and LOP segregates with a 1:1 ratio in the progeny, characterizing maternal gametophytic control. We also show that autonomous embryo formation segregates with autonomous endosperm formation, suggesting that these two loci are closely linked. However, upon meiotic division, embryo lethal components arise and embryo development in apomeiosis mutants was generally defective and seed set was low. Similarly, upon removal of LOP, apomixis initiation occurs normally and unreduced embryo sacs can only form seeds if pollinated.

Autonomous seed initiation is actively repressed in the sexual model plant Arabidopsis by the action of a chromatin remodelling complex encoded by the FERTILIZATION INDEPENDENT SEED (FIS)-class genes. These genes are homologues of the Drosophila PcG complex that also repress gene expression throughout Drosophila development. Mutations in the FIS-class genes lead to elements of apomixis, such as autonomous endosperm, and in one

particular mutant, autonomous egg cell development. Given the similarity in apomictic and FIS-class gene mutant phenotype we isolated three homologues from sexual and apomictic Hieracium plants: *FERTILIZATION INDEPENDENT ENDOSPERM (FIE)*, *MULTICOPY SUPPRESSOR OF IRA1* (*MSI1*) *AND RETINOBLASTOMA* (*RBR*). FIS-class genes from sexual and apomicit *Hieracium* and examined their expression, interaction and function during seed initiation. The isolated *Hieracium* FIS-class genes were highly conserved in sexual and apomictic plants in terms of gene sequence and temporal and spatial expression pattern. Analysis of protein interactions by yeast-two hybrid showed that the HFIE gene from sexual and apomictic plants does not interact with other complex members in the same manner found in Arabidopsis. Protein modelling uncovered structural differences between the Arabidopsis and *Hieracium* FIE proteins. RNAi- mediated down-regulation of *HFIE* in sexual *Hieracium* did not lead to autonomous seed initiation indicating HFIE was not part of a repressive complex. Down-regulation of HFIE in sexual and apomictic plants revealed the gene was essential for embryo growth and viability. Therefore, FIS-complex genes interact differently in Arabidopsis and *Hieracium* and have different developmental roles.

In summary, the results presented here suggest that the FIS-genes are not mutated in apomictic *Hieracium* plants, but they interact differently relative to the Arabidopsis counterparts and play a fundamental role in embryogenesis. Thus, engineering autonomous seed into crops will not depend on mutating these genes but rather in uncovering the molecular signal that triggers apomictic development.

Declaration

This work contains no material that 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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Julio Carlyle Macedo Rodrigues

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Abbreviations

35S – Cauliflower Mosaic Virus 35S promoter

AI – Aposporous initial cell

At – Arabidopsis thaliana

bp – base pairs

BMM – butyl methyl methacrylate

CC – Central Cell

cDNA – complementary DNA

CMT – Chromomethylase

DAPI – 4',6'-diamidino-2-phenylindole

DIG - digoxygenin

DNA – deoxyribonucleic acid

dNTP – 2'-deoxynucleotide triphosphates

EC – Egg Cell

ES – Embryo Sac

FAA – formaldehyde acetic acid

FIE – Fertilization Independent Endosperm

FIS – Fertilization Independent Seed

FPN – Fused Polar Nucleus

GUS – β-D-glucoronidase

H3K – Lysine residue of histone 3

HCl – hydrochloric acid

hp - hairpin

Kb – Kilobase pairs

LOA – Loss of Apomeiosis

LOP – Loss of Parthenogenesis

M- Molar

MEA - MEDEA

MET – DNA methyltransferase, MET1-class

MMC – Megaspore Mother Cell

MSI1 – Multicopy Suppressor of Ira1

mRNA – messenger RNA

ORF – Open Reading Frame

PBS – phosphate buffered saline

PcG – Polycomb Group

PCR – Polymerase Chain Reaction

qPCR – Quantitative PCR

RACE – Rapid Amplification of cDNA Ends

RBR – Retinoblastoma-related Protein

RNA – ribonucleic acid

RT-PCR – Reverse Transcription PCR

SSC – standard saline citrate

UB – ubiquitin

UTR – Untranslated Region

WT – Wild Type