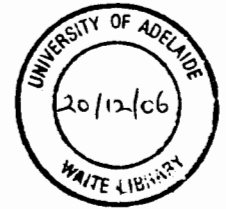


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R2348



Role of the Seed Coat in the Dormancy of Wheat (*Triticum aestivum*) Grains

Submitted by

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This thesis is submitted in fulfilment of the requirements for the degree
Doctor of Philosophy

Discipline of Plant and Food Science
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September, 2006

Table of Contents

<i>Statement of Authorship</i>	<i>ii</i>
<i>Table of Contents</i>	<i>iii</i>
<i>Acknowledgements</i>	<i>ix</i>
<i>Abstract</i>	<i>x</i>
<i>General Introduction</i>	<i>1</i>
<i>Chapter 1: Review of Literature</i>	<i>3</i>
1.1 Pre-Harvest Sprouting in Wheat.....	3
1.2 Economic Impact	3
1.3 Environmental Conditions.....	4
1.4 Spike Morphology	5
1.5 Dormancy in Wheat	6
1.5.1 Dormancy in Red-Grained Wheats	8
1.5.2 Dormancy in White-Grained Wheats	10
1.6 Possible Roles of the Seed Coat Factor in Dormant White-Grained Wheats	11
1.6.1 Structure, composition and function of the wheat seed coat	11
1.6.2 Water uptake	12
1.6.3 Oxygen permeability	16
1.6.4 Chemical Inhibitors in the Seed Coat.....	19
1.6.4.1 Expression of flavonoid biosynthesis genes	21
1.6.5 Chromosomal regions associated with dormancy.....	25

1.7	Summary and Project Aims.....	26
Chapter 2: General Introductory Methods		28
2.1	General Methods.....	28
2.1.1	Plant Material.....	28
2.1.2	Field and glasshouse growing conditions.....	29
2.1.3	Germination testing	30
2.2	Aleurone Samples and Preparation of Seed Coat Material from Developing and Ripe Grains.....	30
2.3	Results	31
2.3.1	Examination of seed coat preparation with fluorescent microscopy	31
2.3.2	HPLC analysis of pure aleurone	33
Chapter 3: Permeability to Oxygen and Effect of Physical Damage to the Seed Coat on Germination and Dormancy		39
3.1	Introduction	39
3.2	Materials and Methods.....	41
3.2.1	Plant material	41
3.2.2	Germination test.....	41
3.2.3	Physical damage to seed coat.....	41
3.2.4	Germination of grain in oxygen-enriched atmospheres	41
3.3	Results	43
3.4	Discussion	47
Chapter 4: Magnetic Resonance Micro-Imaging of Imbibing Wheat Grains		51

4.1	Introduction	51
4.2	Materials and Methods.....	52
4.2.1	Seed Water Uptake.....	52
4.2.2	Imaging of Seed Water Uptake Using MRmI	52
4.3	Results	55
4.3.1	Germination rate for genotypes used in the study.....	55
4.3.2	Physical measurements of grain water uptake.....	55
4.3.3	MR Micro-Imaging of imbibing wheat grains.....	61
4.3.4	MR Micro-Imaging of imbibing wheat grains of differing dormancy.....	64
4.3.4	MR Micro-Imaging of imbibing wheat grains of differing dormancy.....	65
4.3.5	MR Micro-Imaging of grains when access to water was restricted to either the proximal (embryo) or distal (brush) end of the grain	73
4.4	Discussion	75
<i>Chapter 5: Genetic Analysis of a Putative Seed Coat Dormancy Factor.....</i>		80
5.1	Introduction	80
5.2	Materials and Methods.....	81
5.2.1	Genetic material and growing conditions.....	81
5.2.2	Reciprocal crosses and dormancy phenotype of F ₁ grains	81
5.2.3	DNA extraction and QTL analysis.....	81
5.3	Results	83
5.3.1	Dormancy phenotype of F ₁ grains derived from reciprocal crosses.	83

5.3.2	Germination characteristics of doubled-haploid lines.....	83
5.3.3	QTL Analysis.....	90
5.4	Discussion	94
Chapter 6: Expression of Genes Involved in the Flavonoid Biosynthesis Pathway in the Seed Coat of Developing Wheat Grains.....		99
6.1	Introduction	99
6.2	Materials and Methods.....	100
6.2.1	Plant material	100
6.2.2	RNA isolation and cDNA synthesis.....	100
6.2.3	Bioinformatics and primer design.....	101
6.2.4	Reverse Transcription (RT)-PCR.....	105
6.2.5	DNA sequencing	105
6.2.5.1	Gel isolation.....	105
6.2.5.2	PCR purification.....	105
6.2.5.3	Direct sequencing of PCR product with BigDye sequencing reaction .	106
6.2.5.4	Magnesium sulphate cleanup of PCR sequencing reaction.....	106
6.2.6	HPLC analysis of seed coat extracts	106
6.3	Results.....	108
6.3.1	Expression of <i>DFR</i> and <i>CHS</i>	108
6.3.2	HPLC analysis of seed coat extracts	114
6.3.2.1	Seed coat from ripe grains	114

6.3.2.2	Seed coat from developing grains	115
6.4	Discussion	124
<i>Chapter 7: Extraction of Flavonoid Compounds and Potential Germination Inhibitors from the Seed Coat of Dormant Wheat Genotypes..... 130</i>		
7.1	Introduction	130
7.2	Materials and Methods.....	134
7.2.1	Plant material	134
7.2.2	Seed coat extraction and HPLC conditions	134
7.2.3	Standard flavonoid compounds.....	134
7.2.4	Synthesis of apiferol (3)	135
7.2.5	HCl butanol Assay for tannins.....	136
7.2.6	Spiking of alkaline extract of mature seed coat with authentic flavonoids	136
7.2.6.1	Characterisation of extract components	136
7.2.7	Bioassays for potential germination inhibitors in extracts of seed coat....	137
7.2.6.2	Aqueous extract of whole seeds.....	137
7.2.6.3	Aqueous extract from isolated seed coat	137
7.2.6.4	Alkaline extraction of isolated seed coat.....	138
7.3	Results.....	139
7.3.1	Analysis of seed coat extracts.....	139
7.3.2	Determination of tannins in the seed coat of developing and mature grain	142
7.3.3	Alkaline extraction of seed coat.....	149

7.3.4	Spiking of alkaline extracts of seed coat with authentic catechins	158
7.3.4.1	Characterisation of factors in seed coat extracts responsible for losses of added catechin.	159
7.3.5	Effects of seed coat extracts on germination	166
7.4	Discussion	169
Chapter 8: General Discussion.....		175
Bibliography.....		179

Abstract

Pre-harvest sprouting (PHS) is an important economic problem which affects a significant proportion of the Australian wheat crop through quality downgrading. Grain dormancy is the most effective means of overcoming germination in the wheat spikelet at harvest maturity. It has been a consistent observation over a long period of time that dormant red-grained wheat genotypes are almost always more dormant than dormant white-grained genotypes. In white-grained wheat, there are two factors which contribute to dormancy, embryo sensitivity to abscisic acid (ABA) and an interacting and unknown seed coat factor. The proposed dormancy model is that complete dormancy can only be achieved with the coordinated expression of these two factors. This primary objective of this project was to determine the role of this putative seed coat factor in grain dormancy of white-grained wheat.

The physical effect of the seed coat on dormancy was investigated by inflicting damage to the seed coat of Hartog (non-dormant), QT7475 (intermediate-dormant) and SUN325 (dormant) and exposing grains to enriched oxygen conditions. Abrading, slicing and piercing the dorsal seed coat resulted in a loss of dormancy in SUN325 and QT7475, but the increase in germination was similar in both genotypes. The effect of physical damage to the seed coat was therefore not responsible for genotypic differences or the putative seed coat factor. Furthermore, in enriched oxygen conditions germination was also marginally increased in both QT7475 and SUN325, suggesting that the outer seedcoat of imbibed grains reduces the inflow of oxygen, but this increase in germination is not related to the putative seed coat factor.

Magnetic Resonance micro-Imaging (MRmI) was utilised to non-invasively visualise imbibition pathways, primarily to determine if dormant genotypes take up water differently and also to examine general imbibition pathways in the absence of germination. Water uptake followed a structured pathway in all of the genotypes, with the embryo and scutellum tissue hydrating rapidly during early imbibition while the seed coat hydrated more slowly. Water was only able to enter the embryo through the micropyle (a pore through the seed coat in the region of grain attachment to the spike) and there was no observed movement of water across the seed coat and into the endosperm. There was also no observed movement of water beyond the scutellum and into the endosperm in these

early stages of imbibition. The outer layers of the seed coat appeared to conduct water around the exterior of the grain and into the micropyle to hydrate the embryo. Grains of the dormant genotypes did not take up water differently to non-dormant genotypes.

The dormancy model in wheat was validated as a two-component system involving an embryo and an interacting seed coat factor, by performing F_1 reciprocal crosses between a dormant (SUN325), an intermediate-dormant (QT7475) and a non-dormant (Hartog) genotype. The dormancy phenotype was inherited as a recessive trait, with Hartog incurring a loss of dormancy when used as either the male or female parent. Complete dormancy was only achieved in the F_1 progeny when the dormant genotype (SUN325) as the female and QT7475 (intermediate-dormant) as the male parent, indicating that the seed coat from a dormant genotype has an effect on dormancy phenotype. A doubled haploid population of the cross SUN325 x QT7475 was also developed and screened in four different environments over two years, to locate the chromosomal region(s) associated with the seed coat factor. A significant QTL was detected close to the centromere on chromosome 3BL and in a similar region to the *R* genes. These genes are responsible for red seed coat colour.

As a result of the QTL associated with the putative dormant seed coat factor located near the *R* genes, RT-PCR to determine the expression of key genes in the flavonoid biosynthesis pathway was investigated in the seed coat developing wheat grains. A dormant red-grained genotype (R/W635) was also included in the expression analysis of chalcone synthase (*CHS*) and dihydroflavonol 4-reductase (*DFR*), in combination with the white-grained genotypes Hartog (non-dormant), QT7475 (intermediate-dormant) and SUN325 (dormant). At 10 dpa there was no difference in the expression of *CHS* and *DFR*, but at 15 dpa *CHS* was only expressed in the seed coat tissue of R/W635 and SUN325, those genotypes displaying complete dormancy phenotypes. There was no difference in the expression of *DFR* at 15 dpa in all of the genotypes. Subsequent High Performance Liquid Chromatography (HPLC) analysis of the alkaline extract from the seed coat from developing (10 – 30 dpa) and mature grains showed that there was a higher flavone-*C*-glycoside content in SUN325 compared to the other genotypes. This suggests that the longer activation of the flavonoid biosynthesis pathway in SUN325 may result in the production of flavone-*C*-glycosides, while the flavonoid compounds in R/W635 are incorporated into the red pigment, phlobaphene.

Alkaline extracts of the seed coat from seed coat of developing (10 – 30 dpa) and mature grains of white-grained genotypes Hartog (non-dormant), QT7475 (intermediate-dormant), SUN325 (dormant) and the red-grained genotype R/W635 (dormant) was analysed by HPLC to determine if there is a difference in the accumulation of types of flavonoid compounds. Catechin was found to be a minor component of the developing seed coat and present in even smaller quantities in the mature seed coat. When the seed coat extract from the mature grains was spiked with pure catechin and epicatechin compounds, the resultant catechin peak was greatly reduced, even absent, in the HPLC chromatograms from the spiked seed coat extracts of the dormant genotypes (SUN325 and R/W635). This indicates that the catechin/epicatechin compounds are somehow consumed or polymerised with compounds already present in the seed coat extract from mature dormant grains. The consumption of pure catechin/epicatechin compounds may be a clue to the action of the putative dormant seed coat factor.