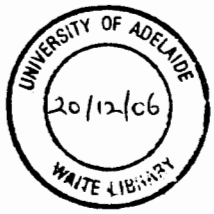

Role of the Seed Coat in the Dormancy of Wheat (*Triticum aestivum*) Grains

Submitted by

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Table of Contents

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.