MACQUARIE UNIVERSITY
FACULTY OF SCIENCE

Biochemical and physiological studies of abscisic acid treated wheat (*Triticum aestivum*) grain

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy by:

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DVDs (Discs 1 and 2) included on back cover:

DVD contents:

Disc 1

- Raw MS data from iTRAQ experiments of the germ tissue (Part 1)

Disc 2

- Raw MS data from iTRAQ experiments of the bran and ventral groove tissues (Parts 2 and 3)
- iTRAQ protein and peptide summaries for the germ, bran and ventral groove tissues
- Supplemental Figures and Tables for Chapter 2
- Mechanical properties analysis (Instron) Supplementary raw data for germ and bran/endosperm

Instructions to open raw MS iTRAQ data (for Parts 1, 2 and 3 only):

Disc 1 – AJerkovic Raw Data. Part 1.exe

Disc 2 – AJerkovic Raw Data. Part 2.rar

Disc 2 – AJerkovic Raw Data. Part 3.rar

To extract this data, copy the three above files from Disc 1 and Disc 2 to the computer's hard drive, double-click on the executable (exe) file and enter a directory for the data to be extracted to (~ 21 Gb uncompressed).

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Declaration

The work presented in this thesis was carried out between January 2007 and June 2011 on a full-time basis. This work represents original research, which has not been submitted for any other degree or to any other University or institution. All work was carried out by the author unless otherwise acknowledged.

Candidates Signature

Ante Jerkovic

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Finally, I thank the GrainFoods CRC for the scholarship and financial support for this project. I have thoroughly enjoyed the experience being part of the GrainFoods CRC.

Chapter 2: This Chapter was published in Plant Physiology in March 2010 and a copy of this publication is attached in Appendix C. The bran tissue collection and 2-DE gels and protein identification was completed by me as part of my Masters thesis. Antibody purification and immunomicroscopy was performed by Alison Kriegel and John Bradner.

Brian Atwell, Thomas Roberts and Robert Willows contributed to writing and editing. All other work in this Chapter and in the publication, which includes collating and interpreting all the information, drafting the manuscript, image analysis and enzyme assay experiments was part of my PhD.

Chapter 7: The MicroMill design and construction – to fulfill the required task for grain fractionation in Chapter 7 – was done by Alan Donald Howard.

Publication and conference posters

Publication

Jerkovic A, Kriegel AM, Bradner JR, Atwell BJ, Roberts TH, Willows RD (2010) **Strategic distribution of protective proteins within bran layers of wheat protects the nutrient-rich endosperm**. *Plant Physiology* 152: 1459-1470

Conference posters

Jerkovic A and Willows RD (2007) **Proteomics of wheat bran.** COMBIO, Sydney Convention and Exhibition Centre, Darling Harbour, Sydney, Australia, 22-26 September 2007.

Jerkovic A and Willows RD (2010) **iTRAQ analysis of abscisic acid treated wheat seeds.** Gordon Research Conference (Salt and Water Stress in Plants), Les Diablerets Conference Center, Les Diablerets, Switzerland, 13-18 June 2010.

Summary

Abscisic acid (ABA) is a well known plant hormone that is involved in many biotic and abiotic stress responses. Application of ABA in the milling of wheat has been shown to improve flour yield and quality. This suggests that there may be biochemical processes which impart a physiological change that is favorable to improving milling performance. In this study, I have attempted to better understand the biochemical and physiological changes of water + ABA-conditioned grain. The strategy involved four stages: (1) apply proteomic analysis to measure and compare the differential expression of proteins in the germ, bran (aleurone layer) and ventral groove of non-conditioned grain (control) and grain conditioned with water-and water + ABA, (2) localize proteins of interest that were identified in the proteomic analysis using immunolabeling and confocal microscopy, (3) test for flour yield and quality by analysing variations in grain fractionation using a laboratory scale mill (MicroMill) to generate fractionation quality scores (FQS's) (4) measure physiological changes in water relations by determining grain water potential (ψ) and mechanical properties of the germ and bran/endosperm of following treatments.

The proteomic analysis of the water-conditioned grain showed that there were many changes in protein levels in the bran and ventral groove tissues, however, there were almost no changes in protein levels in the germ tissue. In the tissues of the ABA-conditioned grain, there were many differentially expressed proteins in the germ, bran and ventral groove tissues, especially those involved in biotic and abiotic stress response. Of these proteins, two are involved in water relations; late embryogenesis abundant proteins (LEA's) and tonoplast intrinsic protein's (TIP's). The ventral groove showed little variation in protein levels between water-and water + ABA-conditioned grain; however, both exhibited a ~ 5-fold increase in LEA's compared to non-conditioned grain. LEA protein abundance also increased in the germ and aleurone cells after ABA treatment but

only by ~ 1.5-fold. Confocal microscopy of immunolabeled grain cross-sections, revealed that group 2 LEA (dehydrin) proteins are distributed throughout the intracellular matrix in a 'honeycomb-like' arrangement and also surrounding the nucleus and inner cell walls within the germ cells, aleurone cells in the bran layer and in the aleurone cells surrounding the ventral groove. TIP levels decreased by ~ 2-fold exclusively in the germ, which is likely to reduce water movement in and out of the tonoplast. An increase in FQS's of ABA-conditioned grain compared with water-conditioned grain may indicate improved fractionation, leading to improved flour quality and yield. Psychrometric measurements of the germ-end and bran/endosperm-end of ABA-conditioned grain showed a slightly elevated ψ when compared to water-only treatment after drying at room temperature for 1.5 h. This suggested that the grain somehow retains more moisture if treated with ABA. Finally, mechanical property analysis of the germ and bran tissue showed that the germ was softer and bran was tougher after conditioning with ABA.

Collectively, these results suggest that ABA may induce changes in biochemical processes of the germ and aleurone cells of the bran and ventral groove tissues, such as increased levels of LEAs and a reduction in TIPs in order to prevent moisture loss in response to an environmental stress such as drought. Consequently it is suggested that this altered the water distribution within the grain, thus transforming its physiological properties, making it better adapted to surviving a desiccating environment. From an applied science perspective, this physiological change allows the grain to be more amenable to milling, thus improving flour yield and quality.

Abbreviations

ABA	Abscisic acid
CID	Collision induced dissociation
1-DE	One-dimensional electrophoresis
2-DE	Two-dimensional electrophoresis
DIC	Differential interference contrast
DPA	Days post anthesis
EST	Expressed sequence tag
FBS	Fetal bovine serum
FQS	Fractionation quality score
GA	Gibberellic acid
TheGPM	The Global Proteome Machine
iTRAQ	Isobaric tags for relative and absolute quantification
LC	Liquid chromatography
LEA	Late embryogenesis abundant
MALDI	Matrix assisted laser desorption ionisation
FQS	Fractionation quality score
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
ROS	Reactive oxygen species
RT	Room temperature
SDS	Sodium dodecyl sulfate
TOF	Time of flight