The genetic improvement of wheat and barley for reproductive frost tolerance

By

Jason Reinheimer

Bachelor of Agricultural Science, University of Adelaide

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Sciences at the University of Adelaide

School of Agriculture, Food and Wine
Waite Campus

2010

Table of Contents

Abstract

The aim of the research reported in this thesis was to identify genetic variation for Reproductive Frost Tolerance (RFT) in barley, characterise the genetic basis of the observed tolerance and devise and execute a strategy to incorporate the tolerance into germplasm adapted to Australian production environments. Effects of wheat chromosome regions syntenous to the barley RFT loci were also investigated.

A field based screening nursery was developed to characterise barley germplasm for RFT. A diverse collection of international barley germplasm was screened for RFT to identify barley genotypes exhibiting better levels of RFT than what was available in Australian cultivated germplasm. Three lines were identified as having an increased level of RFT and populations derived from these three lines were used to QTL map RFT traits. One QTL was common between the three populations and a second QTL was common between 2 of the populations. These two loci were found to control a reduction in Frost Induced Sterility (FIS) and frost induced grain damage.

One of the barley QTL spanned the vernalisation response gene and vegetative frost tolerance locus *vrn*-H1/*Fr*-H1. The syntenous genomic regions in hexaploid wheat were investigated to determine if they had an effect on RFT. Two sets of wheat germplasm containing variation for winter/spring alleles of *vrn*-A1, *vrn*-B1 and *vrn*-D1 loci revealed that no measurable differences in RFT were associated with these loci in wheat.

Targeted populations were developed from selected tolerant and intolerant genotypes. The location of the RFT locus on chromosome 5HL in barley was refined further by developing molecular markers within the QTL region. A population phenotyped for RFT using a frost

simulation chamber revealed that the RFT locus on chromosome 5HL was distal to the major phenology gene *vrn*-H1 segregating in the population.

A breeding strategy was devised to rapidly incorporate the two RFT loci into Australian adapted barley that utilised specific germplasm, newly developed FIS phenotyping methods, molecular markers and doubled haploid technology. A recurrent parent was selected based on adaptation to regions that experience frequent damaging frost events. Germplasm derived from this breeding strategy was screened with diversity array technology markers to determine the effect of donor introgression segment size on yield in target environments.

The resulting germplasm from the fast track breeding strategy was evaluated for RFT using field and controlled environment frost tolerance screening methods. The adaptation of the developed germplasm was assessed by conducting yield trials in the environments where frost is a major risk to production in southern Australia. The performance of this germplasm under a range of frost events provided a better understanding of the conditions in which this source of tolerance is effective.

The results of this thesis highlighted challenges associated with RFT breeding in barley and wheat. The systematic way in which this work was approached through the development of screening methods, identification of genetic variation, genetic analysis and devising a breeding strategy to incorporate the tolerance into adapted germplasm provides a good example of how a cereal crop can be rapidly improved for a quantitative trait such as RFT.

Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Reinheimer JL, Barr AR, Eglinton JK (2004) QTL mapping of chromosomal regions conferring reproductive frost tolerance in barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics 109, 1267-1274.

Jason Reinheimer - 2010

Acknowledgements

Over the past 6 years completing this PhD part time while working a fulltime job has required much support and encouragement from many people and to all I am very grateful.

I am particularly grateful for the assistance provided by the University of Adelaide barley lab where I spent much of the time completing this research and who continued to support me once I moved into breeding. My particular thanks go to Stewart Coventry, Nigel Long, Calum McCafferty and Helen Collins. Very special thanks must go to Michael Laws who has completed much of the grunt work on this project. I would also like to thank the University of Adelaide barley breeding field team who were always 'happy' to accommodate my numerous requests for assistance with field work. Thanks to Andy Barr who took a chance on a graduate and employed me straight out of University to run a research project with no experience. I owe much of my interest in plant breeding to Andy's lectures in undergraduate studies.

Thankyou to the members of the map based cloning lab at the Australian Centre for Plant Functional Genomics. My particular thanks to Andrew Chen and Anita Brule-Babel for their help and support on this project.

I would like to thank all of the staff at the Roseworthy AGT node for their understanding and help during the completion of this thesis. They have been the people who had to pick up the 'slack' with my real job while I worked on my PhD. I would particularly like to mention the assistance of Haydn Kuchel. The discussions we have had on not just our PhD projects but also all aspects of breeding and research have and continue to be very enjoyable.

A special mention needs to go to Steve Jefferies. Allowing me to complete a PhD while working as a plant breeder has been a dream. I am very grateful Steve.

To my supervisor Nick Collins, thanks for spending the time with a very anti bench work person to help me enjoy the craft of the 'gene jockey'. You are probably the person I have learnt the most from while completing my PhD project in an area I would otherwise have not been exposed to. The time and patience you have put in reviewing my thesis and improving my writing is much appreciated.

To my primary supervisor Jason Eglinton, thanks mate. Your ability to give me freedom to manage this research while providing guidance when required has been amazing. Much of my development has come from your guidance. I will certainly enjoy the beer I have with you when this is all over.

To my parents, Trish and Ivan, thanks for always supporting me in whatever I have chosen to do. I think anyone who works in this industry needs to spend some time working on a farm like I have had the pleasure of doing at home. It certainly makes you appreciate what you are working for and puts everything into perspective.

To my wife Nicole, thanks for always being there when I have needed your support over this long journey. I am going to have to come up with another excuse now to get out of doing the washing and cleaning. I am looking forward to spending the extra time I will have with you.

Statement of Author Contributions

Paper Title:

Reinheimer JL, Barr AR, Eglinton JK (2004) QTL mapping of chromosomal regions conferring reproductive frost tolerance in barley (Hordeum vulgare L.). Theoretical and Applied Genetics 109, 1267-1274.

Jason Reinheimer (Candidate)

Designed experiment, conducted research, analysed and interpreted data, wrote manuscript and acted as corresponding author.

Signed [redacted] Date 25/01/10

Jason Eglinton

Project supervisor and assisted with preparation of manuscript

I give consent for Jason Reinheimer to present this paper for examination towards the Doctor of Philosophy

Signed [redacted] Date 27/1/10.

Andrew Barr

Project supervisor and assisted with preparation of manuscript

I give consent for Jason Reinheimer to present this paper for examination towards the Doctor of Philosophy

Signed [redacted] Date 27/1/2010