Dissection of Traits Relating to Flowering and Reproductive Frost Tolerance on Chromosome 2HL of Barley (*Hordeum vulgare* L.)

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B. Btech (Hon)

Submitted to the University of Adelaide for the degree of Doctor of Philosophy

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November 2008

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Abstract

In Australia, cereal crops such as wheat and barley are planted in autumn with the majority of the growing season occurring over winter. This results in occasional exposure of cold sensitive reproduction organs of the florets to frost events (-2 to -4°C) that sporadically occur in winter and early spring. Direct frost damage to cereal reproductive tissues can cause up to 85% yield losses and is estimated to cause 10% reduction in long-term yield in Australia. Two loci (2H and 5H) controlling frost tolerance at the reproductive stage were identified in Amagi Nijo × WI2585 and Galleon × Haruna Nijo DH populations, with alleles inherited from the Japanese parents associated with tolerance. The 5H locus (*Fr-5H*) position is close to the *Triticeae* homoeoloci influencing vernalization response (*Vrn-1*) and vegetative frost tolerance (*Fr-1*), while no frost tolerance effects had previously been reported in the region of the 2H locus (*Fr-2H*) in cereals. In the current study, the 2H and 5H chromosome regions controlling frost tolerance were also found to control developmental traits (e.g. flowering time), suggesting that developmental effects could directly or indirectly determine frost tolerance at one or both loci. However, preliminary data suggest that none of the developmental traits were consistently associated with tolerance. Using rice-barley co-linearity, the flowering time effect on 2HL (we named *Flt-2L*) was delimited to a 1.3 cM genetic interval in barley where it co-segregated with flowering time, spike compactness, plant height and an *APETALA2*-like gene. The *AP2* gene represents a plausible candidate for *Flt-2L* because members of the *AP2* gene family have been shown to control flowering time in maize, rice and wheat. Further analysis showed that the 2H frost tolerance effect can be genetically separated from *Flt-2L* by recombination. Thus frost tolerance at this locus appears to be controlled by a tolerance *per se* mechanism and is not as a result of flowering time differences (frost escape). Therefore, tolerance is unlikely to be due to a pleiotropic effect of *Flt-2L*. Floret sterility levels obtained using a frost simulation chamber distinguished the parents and F_2 derived individuals carrying contrasting alleles at the 2H tolerance locus. The use of an ice nucleator facilitated uniform freezing on the surfaces of the spikes and leaves, and was used to demonstrate that the 2H effect likely depends on freezing and not chilling. Future activities will include using rice-barley co-linearity to isolate the gene(s) responsible for frost tolerance at the 2H and 5H loci. The emerging physical maps of barley and wheat and the genome sequence of *Brachypodium* will accelerate the positional cloning. Candidate genes will be functionally analyzed using both forward and reverse genetic approaches. Markers linked to the genes controlling tolerance will be given to breeders to assess the value of the tolerance alleles in the field.

Declaration

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.

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Andrew Chen
November 2008

Acknowledgement

I gratefully acknowledge my supervisors, Dr. Nik Collins, Dr. Ute Baumann and Professor Geoffrey Fincher, for their unwavering guidance, support and advice during the past three years. I am especially thankful to each of them for the enthusiasm and professionalism displayed in supervising my project. Their outstanding approach allowed me to develop new ideas and test new theories which paved the way for me to explore the project the way I have. In particular, I would like to thank Nick for his devotion to the overall supervision of this project and the enormous amount of time he has spent in getting the paper manuscripts ready for publication. His commitment and support played a major part in allowing me to finish my Ph.D. project and thesis on time. I would like to extend my warm thanks to Professor Larry Gusta and Dr. Richard Leach for their significant inputs in the frost project which could not have progressed the way it has without their deep understanding in plant physiology and freezing. I wish to thank the Australian Centre for Plant Functional Genomics and Professor Peter Langridge for providing me with the Ph.D. scholarship to carry out the scientific research. I gratefully acknowledge our funding bodies, the Grains Research and Development Corporation, the Australian Research Council, the University of Adelaide and the South Australian government.

In particular, I would like to extend my thanks to Ursula Langridge and Robin Hoskin for their outstanding commitments in greenhouse and growth room maintenance. I would also like to thank Dr. Bujun Shi for assistance, pep talks, opinions and comments on various aspects of my project throughout the year. I would like to thank the past and present members of the map-based cloning group, including Tim Sutton, Alison Hay, Alireza Rivandi, Chunyuan Chuang, James Edwards, Joanne Eldsen, Nadim Shadiac, Filippo Bassi and Thorsten Schnurbusch for their input, advice, friendship and technical help in particular areas of this work. I am also very appreciative for the oppoturnity to interact with everyone at the Australian Centre for Plant Functional Genomics, for it was certainly a joyful experience during my time here.

Finally, I would like to thank all my family (e.g. Jisuk, mum and dad) and friends for their unwavering support, patience and understanding.