

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

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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 F₂ 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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- Chen A, Brûlé-Babel A, Baumann U and Collins NC (2008) Structure-function analysis of the barley genome: the gene-rich region of chromosome 2HL. *Funct Integr Genomics* (In press)
- Chen A, Reinheimer JL, Brûlé-Babel A, Baumann U, Fincher GB and Collins NC (2009) Genes and traits associated with barley 2H and 5H chromosome regions controlling sensitivity of reproductive tissues to frost. *Theor Appl Genet* (Revised submission)
- Chen A, Baumann U, Fincher GB and Collins NC (2009) *Flt-2L*, a locus in barley (*Hordeum vulgare* L.) controlling flowering time, spike density and plant height. *Funct Integr Genomics* (In press)
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