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QTL mapping of chromosomal regions conferring reproductive frost tolerance in barley (*Hordeum vulgare* L.)

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Abstract Spring radiation frost is a major abiotic stress in southern Australia, reducing yield potential and grain quality of barley by damaging sensitive reproductive organs in the latter stages of development. Field-based screening methods were developed, and genetic variation for reproductive frost tolerance was identified. Mapping populations that were segregating for reproductive frost tolerance were screened and significant QTL identified. QTL on chromosome 2HL were identified for frost-induced floret sterility in two different populations at the same genomic location. This QTL was not associated with previously reported developmental or stress-response loci. QTL on chromosome 5HL were identified for frost-induced floret sterility and frost-induced grain damage in all three of the populations studied. The locations of QTL were coincident with previously reported vegetative frost tolerance loci close to the *vrn-H1* locus. This locus on chromosome 5HL has now been associated with response to cold stress at both vegetative and reproductive developmental stages in barley. This study will allow reproductive frost tolerance to be seriously pursued as a breeding objective by facilitating a change from difficult phenotypic selection to high-throughput genotypic selection.

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Introduction

Spring radiation frost affects grain production across a significant area of the Australian cereal belt. In southern Australia, barley is planted in autumn with the majority of the growing season over winter. Winter temperatures are not low enough to cause considerable frost damage to barley at the vegetative stage of development during these months. The predominant frost damage occurs from radiation frost events in spring during the reproductive stages of barley and other cereals' development. Radiation frosts occur under clear night skies, where more heat is radiated away from the crop canopy than it receives. The loss of radiant energy causes the temperature to fall, which can damage sensitive reproductive tissue at sub-zero temperatures (≤2°C). These frost events can cause floret and spike abortion as well as damage to the developing grain, which can have a significant impact on yield and quality.

Since the 1930s, attempts have been made to identify genetic variation for spring radiation frost tolerance in wheat and incorporate the associated genes into commercially relevant adapted material with very limited success (Marcellos 1988). The unreliability of previous field-based screening methods from confounding factors such as spatial variation, seasonal variation and overriding maturity effects has made progress slow (Single 1988).

In contrast, genetic variation for vegetative frost tolerance has been exploited for many years within a number of crops, including wheat and barley, and more recently, there has been an improved understanding of its genetic control (Sutka 2001; Hayes et al. 1993a). Using field survival data from Oregon and Montana (USA) and lethal temperature resulting in 50% mortality (LT50) values, using a temperature-controlled growth cabinet, Hayes et al. (1993a) mapped the genetic location of genes controlling vegetative frost tolerance in a doubled haploid mapping population of barley between the frost-tolerant cultivar Dicktoo and frost-susceptible cultivar Morex. Hayes et al. (1993a) proposed that a QTL for all cold tolerance measures were found on barley chromosome 7(5H). The group 5

chromosomes in wheat have also been identified as having significant effects on vegetative frost tolerance. Map locations of traits associated with vegetative frost tolerance have been reported on syntenous regions of chromosomes 5A, 5D (Sutka 2001) and 5B (Toth et al. 2003), which are homoeologous to the 5H frost tolerance/vernalisation region in barley (Snape et al. 2001).

A relationship between vegetative and reproductive frost tolerance has not been previously observed. It has been reported that the ability of a cereal to cold harden significantly increases its vegetative frost tolerance, something that is lost after the commencement of differentiation of the shoot apex (Single 1988). The aim of the current study is to identify genetic variation for spring radiation frost tolerance in barley and to identify chromosomal regions associated with increased tolerance, using populations derived from frost-tolerant × frost-susceptible crosses.

Materials and methods

Genetic material

A diverse collection of germplasm—targeting areas that experienced damaging spring and winter frost events—was assembled. Germplasm was sourced from commercial varieties, breeders' lines, landraces and wild barley introgressions. Source countries included Afghanistan, Algeria, Australia, Austria, Ecuador, France, Germany, Iran, Japan, Mexico, Nepal, Netherlands, Pakistan, Sweden, Syria, Turkey, the United Kingdom and the United States.

The germplasm used for the mapping of frost tolerance loci was derived from three mapping populations consisting of: (1) a mapping population of 225 doubled haploid lines derived from a cross between the Australian varieties Franklin and Arapiles (Verbyla et al. 2003); (2) a mapping population of 112 doubled haploid lines derived from a cross between the Australian variety Galleon and the Japanese variety Haruna Nijo (Karakousis et al. 2003) and (3) a mapping population of 139 doubled haploid lines derived from a cross between the Japanese variety Amagi Nijo and the Australian (Waite) breeders line WI2585 (Pallotta et al. 2003).

Field screening for reproductive frost tolerance

Two spring frost-prone sites at Loxton (latitude: −34.4333°S, longitude: 140.6000°E, elevation: 66.0 m) and Black Rock (latitude: −32.8333°S, longitude: 138.7000°E, elevation: 596.0 m) in South Australia were selected to sow reproductive frost tolerance-screening nurseries. Early seeding times in autumn (March/April compared to 'normal' of May/June) were used to encourage early flowering during the peak frost-risk period (July/August). Irrigation was used to promote early growth and to supplement rainfall events during the growing season. Four seeding times were required to allow for maturity differences between genotypes and to collect data from multiple frost events during the season. Each genotype was replicated in a 1.5-m row. This method of seeding was used to give the maximum number of genotypes in a small area to reduce the effect of spatial temperature variation. Thermocouples were distributed throughout the trial area at a height of 80 cm (average spike height) to identify spatial differences. An alarm was triggered after each frost event (critical temperature = −2.0°C) and relayed via remote telemetry to a mobile phone. Directly after a frost event, 10 tillers/entry that were at the same developmental stage were tagged so direct comparisons between genotypes could be made. Just prior to anthesis [decimal growth stage (DGS) 59 and early grainfill (DGS73); Zadoks et al. 1974], the developmental stages were targeted so different developing tissue could be compared. Frost-induced sterility (FIS) was assessed 10–20 days later on each spike tagged with damage scores calculated on the number of sterile florets as a percentage of total florets that had potential to fill grain. Frost-induced grain damage (FIGD) scores were taken at physiological maturity.

During the 2001 season, 100 diverse genotypes were planted in the frost nurseries at Loxton and Black Rock. Lines that performed significantly better than current Australian adapted commercial varieties were tested again in the 2002 season.

Collecting phenotypic data

The Galleon/Haruna Nijo and Amagi Nijo/WI2585 mapping populations were included in the 2002 frost-screening nursery at Loxton. FIS data were collected from both populations for QTL mapping. Physiological traits collected on the Amagi Nijo/WI2585 population included height to flag leaf, height to base of spike, peduncle length and DGS on primary tillers, secondary tillers and tillers tagged for frost-damage scoring.

The Arapiles/Franklin mapping population was grown in a randomised complete block design with two replicates at Horsham, Victoria, by David Moody, DPI Victoria. Each entry was sown in a 6-m, six-row (150-mm spacing) plot on 29 June 2002. FIS and FIGD scores were taken at physiological maturity.

Data analysis

Results collected from each frost event were subjected to the analysis of variance (ANOVA) statistical analysis to determine the significance of the genotypic effect on the level of frost damage. Genotype frost damage means and least significant differences of means were calculated via the ANOVA model.

The program Qgene, version 3.04, was used for mapping frost-damage data. Simple and interval regression

analysis were used for determining significant trait-marker associations, with a minimum LOD threshold of 3.0.

Results

Frost events

During the 2001 season, a frost event on 30 June caused extensive damage to reproductive tissue of genotypes in the developmental range of DGS49 (awns peeping) to DGS83 (early dough) (Zadoks et al. 1974). Canopy (80 cm above ground) minimum temperatures of −4.6°C and −3.0°C were recorded at Loxton at Black Rock, respectively, allowing for discrimination between tolerant and susceptible genotypes. On 11 October 2001, a frost event at Horsham was recorded, where the minimum temperature reached −1.0°C (screen temperature at 1.5 m above ground). Damage to floral tissue and developing grain of barley lines grown at Horsham resulted from this event.

During the 2002 season, two discriminating frost events were recorded at Loxton. On 2 July, a minimum canopy temperature of −4.5°C was recorded. On 21 July, a minimum canopy temperature of −2.8°C was recorded. Both events caused damage to reproductive tissue of genotypes post-spike emergence.

Screening for genetic variation in reproductive frost tolerance

The initial ANOVA of raw percentage sterility data from Loxton 2001 identified a significant ($P<0.001$) genotype effect on sterility. The raw data were not normally distributed and were therefore transformed using LOG_{10} (sterility + 2.5) to obtain a more normal distribution of values. The ANOVA using the converted data also demonstrated a significant ($P<0.001$) genotype effect on FIS. A number of genotypes exhibited significantly less FIS than Australian commercial varieties after the damaging frost event (Fig. 1). The Japanese varieties Haruna Nijo (4.5%) and Amagi Nijo (5.4%) had significantly lower FIS than Australian commercial varieties including Arapiles (27.2%), Galleon (40.3%) and Schooner (79.1%).

Raw data collected from the Loxton frost-screening nursery in 2002 were also converted using a log term [LOG_{10} (sterility + 1.5)] to gain a more normal distribution of the data. From the ANOVA using the converted data, a significant ($P<0.001$) genotype effect on FIS was observed. The FIS results from the 2002 frost screening produced results similar to 2001, with Haruna Nijo (10.3%) and Amagi Nijo (6.1%) again performing significantly better than Australian commercial varieties Arapiles (57.1%), Galleon (62.8%) and Schooner (39.1%) (Fig. 2). The Franklin sib line also performed well in 2002, with a FIS of 11.4%.

QTL mapping of reproductive frost tolerance

FIS and FIGD were analysed in the Arapiles/Franklin mapping population. A third term was used in the analysis of the population that was a combination of the two scores and named total frost-induced damage (TFID). Data collected were normally distributed and therefore, no transformation was employed. The ANOVA indicated a significant genotype effect on FIS (0.01), FIGD (>0.001) and TFID (>0.001). Using the means for each genotype in the population, significant QTL were identified for FIS and FIGD, with both located in the same region on 2HS. A QTL effect was also evident for FIS on 3HL but not for FIGD (data not shown). Both these regions have been identified as being significantly associated with maturity in this population. The QTL identified on 2HS were located at the same locus as the earliness per se gene [(*eps*2) Laurie et al. 1995]. The QTL on 3HL mapped to the same region as the *denso* dwarfing gene (Laurie et al. 1995). Frost-damage scores mapping to maturity loci indicate a likely frost-escape effect rather than a true tolerance effect being observed in the QTL analysis. Statistically non-significant QTL were detected for FIGD (LOD=2.5) and TFID (LOD=2.9) on chromosome 5HL. These non-significant QTL were further investigated using a sub-set of 101 lines with a narrow maturity range to reduce the maturity effect on frost damage. Using lines in the maturity range of early milk (DGS73) to late milk

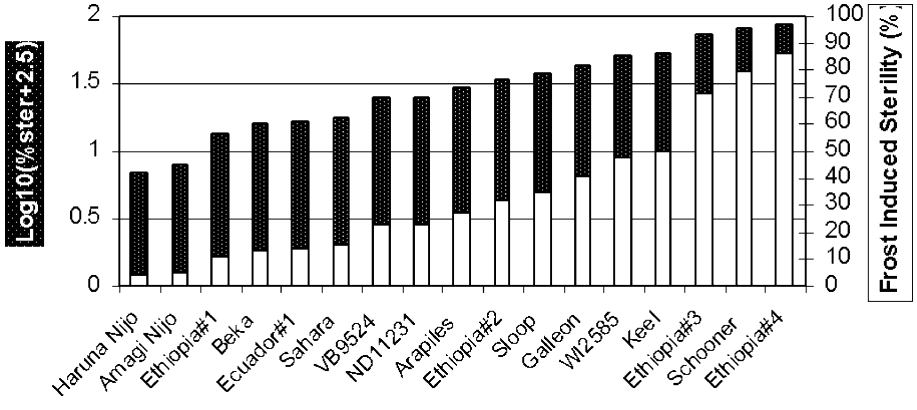

Fig. 1 Frost-induced sterility (FIS) observed on barley lines during the 2001 season at Loxton, South Australia. LSD=0.5 and relates to the log-converted data (*left axis*). Raw percent FIS represented by the *white bars* on the *right axis*

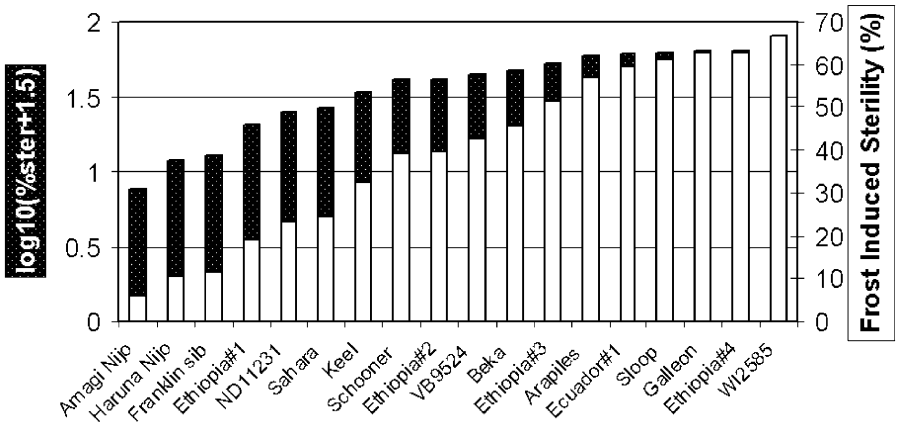

Fig. 2 FIS observed on barley lines during the 2002 season at Loxton, South Australia. LSD=0.2 and relates to the log-converted data (*left axis*). Raw percent FIS represented by the *white bars* on the *right axis*

(DGS77) (Franklin: DGS71, Arapiles: DGS83), the QTL for FIS and FIGD on chromosomes 2HS and 3HL became non-significant, whereas the significance of QTL on 5HL for FIGD and TFID increased. The QTL for FIGD increased to a LOD score of 3.9 and TFID to a LOD score of 4.3. The QTL on 5HL was associated with SSR marker Hv635P2.4f and accounted for 19% and 20% of the observed phenotypic variation for FIGD and TFID respectively (Table 1). The Arapiles allele was associated with the higher damage score and therefore, the Franklin allele at this region contributed to the increased tolerance to frost. FIS was measured in the Amagi Nijo/WI2585 population at Loxton during the 2002 season. The ANOVA using the FIS data indicated a significant genotype effect in the Amagi Nijo/WI2585 population for both frost events (0.001). Using the means from the Amagi Nijo/WI2585 population after the first frost event, a significant QTL for FIS was mapped to chromosome 5HL (Table 1). No other significant QTL were identified from the first frost event in this population. From the data collected from the second frost event at Loxton, 2002, two significant QTL were identified. The first was located on chromosome 2HL, which had a LOD score of 3.5, and the second was located on chromosome 5HL, with a LOD score of 4.7 (Table 1). The two significant markers on 2H and 5H were tested for a two-way interaction for the FIS trait. No significant (P>0.05) interaction was identified. Both QTL on 5HL for FIS were located in a similar position on the chromosome, with markers BCD265a and ABG702b 13.8 cM apart. In all cases, the WI2585 allele was associated with the higher FIS score.

A significant genotype effect on FIS (P<0.001) was observed at Loxton for both frost events during the 2002 season. However, no significant QTL were identified for FIS from the Haruna Nijo/Galleon population after the first frost event. From the second frost event, two QTL were identified for FIS. A QTL on 2HL was significantly associated with the trait, with a LOD score of 5.0. The second significant QTL was identified on chromosome 5HL, with a LOD of 3.2 (Table 1). For both QTL, the Galleon allele was associated with the higher FIS score.

QTL mapping physiological and morphological traits

In all three mapping populations used in this study, segregation for potential confounding effects such as plant height, peduncle length and maturity may have contributed to avoidance rather than true tolerance to frost. To investigate these effects, the Amagi Nijo/WI2585 population was used to take detailed physiological and morphological measurements.

Significant QTL were identified for the primary tiller DGS. The first, on chromosome 2HS, was located in a similar region to the *eps2* developmental locus (Fig. 3). This locus was proximal to the frost tolerance locus on chromosome 2HL. The second, on chromosome 5HL, was located in the same region as the vernalisation response (*vrn-H1*) developmental locus (Fig. 4).

This locus was also implicated in tolerance to FIS in the two Japanese populations, indicating a possible avoidance rather than tolerance effect being observed as the level of susceptibility of barley varies significantly from pre- to

Table 1 QTL analysis of frost-induced grain damage (*FIGD*), total frost-induced grain damage (*TFID*) and frost-induced sterility (*FIS*) in the mapping populations Arapiles/Franklin (*A/F*), Galleon/Haruna Nijo (*G/HN*) and Amagi Nijo/WI2585 (*AN/WI*) grown at Horsham, Victoria, and Loxton, South Australia, in 2001 and 2002

Population	Trait	Location	Date	Chromosome	Marker	LOD	r^2	Parent
A/F	FIGD (SS)	Horsham	11 Sep 2001	5H	Hv635P2.4f	3.9	0.19	Arapiles
A/F	TFID (SS)	Horsham	11 Sep 2001	5H	Hv635P2.4f	4.3	0.20	Arapiles
G/HN	FIS	Loxton	21 Jul 2002	2H	HVM54	5.0	0.42	Galleon
G/HN	FIS	Loxton	21 Jul 2002	5H	AWBMA13b	3.5	0.44	Galleon
AN/WI	FIS	Loxton	02 Jul 2002	5H	MWG514	5.9	0.32	WI2585
AN/WI	FIS	Loxton	21 Jul 2002	2H	HVM54	3.4	0.18	WI2585
AN/WI	FIS	Loxton	21 Jul 2002	5H	BCD265a	4.5	0.26	WI2585

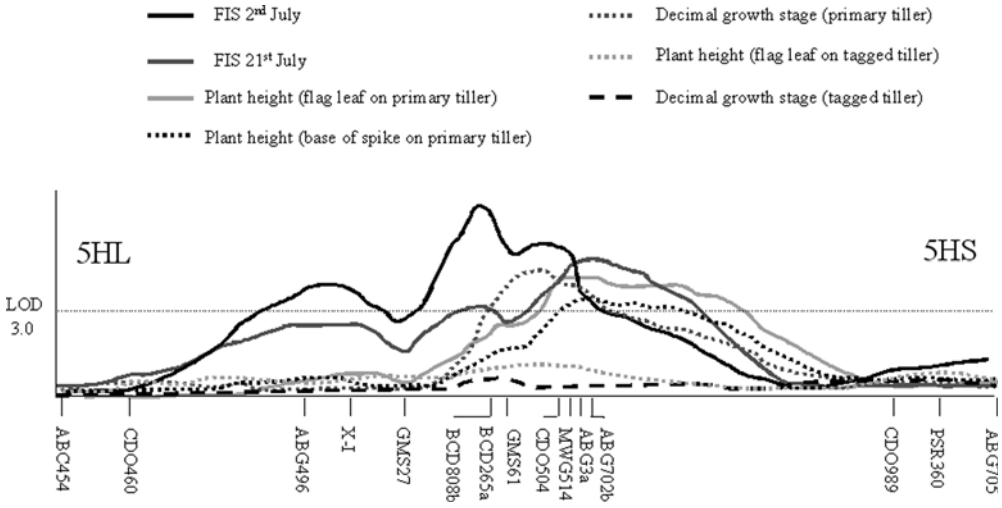

Fig. 3 Coincidence of QTL for FIS, plant height and decimal growth stage (DGS) on chromosome 5H in the Amagi Nijo/WI2585 mapping population, based on data collected from Loxton, South Australia, in 2002

post-anthesis. Tagging heads of the same developmental stage directly after a frost event was aimed to overcome this confounding effect. To validate this method, developmental scores were recorded on tagged heads from which FIS was recorded and used in the QTL analysis. No QTL were identified indicating that maturity was removed as a confounding effect on FIS by the method of tagging heads at a discrete developmental stage (Fig. 4).

As temperature can vary significantly over different canopy heights during a radiative frost event, plant height can also have a confounding effect on frost tolerance. It has been reported that within a wheat canopy during a radiative frost event, the coldest point is at the head height, with a temperature gradient above and below this level (Single 1988). Therefore, both plant height and peduncle length may have confounding effects on true tolerance. Height to flag leaf and to the base of the spike on the primary tiller mapped to the same relative location on chromosome 5HL as FIS. When these two traits were mapped using scores from tillers used for FIS scoring, no significant QTL were identified (Fig. 4). This indicates that plant height was unlikely to have a significant effect of the FIS data.

Although all three populations were crosses between spring parents that did not respond to vernalisation, both Japanese crosses were segregating for this trait. Phenotypic data for response to vernalisation treatments collected on the Galleon/Haruna Nijo population (W.J.R. Boyd, unpublished data) was analysed for interactions between combinations of alleles at each of the three *Vrn* loci. Marker alleles linked to *Vrn-H1* and *Vrn-H2* on chromosomes 5H and 4H, respectively, showed a significant interaction ($P<0.001$). All genotypes in the population with Haruna Nijo alleles at *Vrn-H1* and Galleon

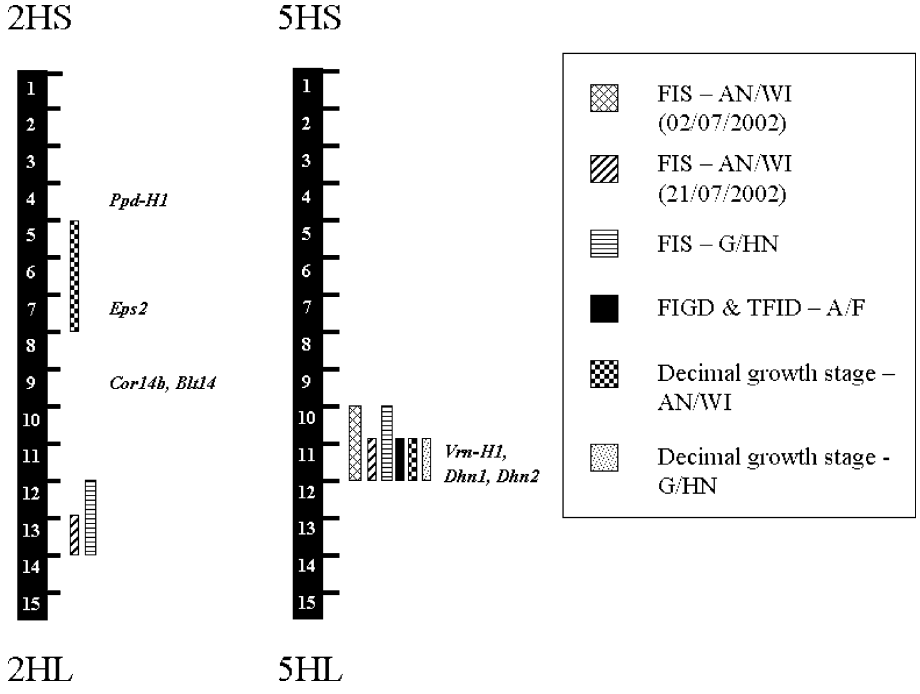

Fig. 4 A schematic bin map (Kleinhofs and Han 2002) of barley chromosomes 2H and 5H, showing locations of QTL for FIS, FIGD, TFID and DGS for the Australian mapping populations Amagi Nijo/WI2585 (*AN/WI*), Galleon/Haruna Nijo (*G/HN*) and Arapiles/Franklin (*A/F*). QTL (LOD>3) are represented by *vertical bars*. Candidate developmental and cold-regulated gene loci are in *italics*, positioned based on reported locations (Laurie et al. 1995; Cattivelli et al. 2002a)

alleles at *Vrn-H2* responded to vernalisation. No genotypes with other combinations of alleles responded to vernalisation.

Discussion

Source of genetic variation in reproductive frost tolerance

The Japanese varieties Haruna Nijo and Amagi Nijo were both bred by Sapporo Breweries, Japan. The growing conditions of barley in Japan is characterised by low temperatures during the early vegetative growth stages. Cold-induced leaf damage and plant death is common. Genetic variation for tolerance to these growing conditions has been observed, with Haruna Nijo and Amagi Nijo more tolerant, compared to other genotypes and in particular, Australian barley varieties (K. Ogushi, personal communication). Both varieties have similar genetic backgrounds with vernalisation responsive genotypes in their pedigrees (Kihara et al. 1998). Genes that promote winter growth habit are common in Japanese germplasm. Moderate winter habit prevents damage to young spike primordia through the cold Japanese winter months (Takahashi and Yasuda 1971). The major vernalisation responsive gene *vrn-H1* in barley is believed to be closely linked to winterhardiness traits at the vegetative stage of barley development (Hayes et al. 1993a). This is the most likely source of tolerance in Haruna Nijo and Amagi Nijo. Franklin is an Australian-bred long-season malting variety. Franklin was derived from a cross between the Australian variety Shannon (Proctor*4/CI3208-1) and the German variety Triumph (Diamant/St 1402964/6). It is unclear from where the tolerance has been derived, as there is little data available on the characteristics of some of the parental lines. It is possible to speculate on the source of frost tolerance by considering the breeding and selection of lines in Franklin's parentage. CI3208-1 is an Ethiopian-derived line in Shannon's pedigree. Much of the area growing barley in Ethiopia has periods of extreme cold at later stages of barley development. The reproductive frost tolerance of material derived from Ethiopia is supported by the observed tolerance of line Ethiopia #1, screened in the Loxton frost nursery in 2001 (Fig. 1) and 2002 (Fig. 2).

Further genotyping and phenotyping work is needed to identify the original source of reproductive frost tolerance in these varieties.

QTL mapping of reproductive frost tolerance

Mapping data collected from the three populations in this study indicated that two chromosomes, 2H and 5H, were implicated in the genetic control of the reproductive frost tolerance traits. Using a bin-map system, based on the Steptoe/Morex population (Kleinhofs and Han 2002), the relative location of QTL in the three different populations was identified (Fig. 4).

QTL identified in the Amagi Nijo/WI2585 and Galleon/Haruna Nijo populations that mapped to chromosome 2HL were located in the same bin location (Fig. 4). This locus in both populations was independent of all other traits mapped including developmental and other stress-response traits. In other mapping populations, few traits have been identified as significantly associated with this bin location on 2HL. These traits include ear weight, thousand-grain weight and hot-water extract from the Blenheim/Kym population (Bezant et al. 1997), height and lodging from the Steptoe/Morex population (Hayes et al. 1993b) and screenings from the Sloop/Alexis population (Coventry et al. 2003). Although these traits reported in bin 13 can be affected by developmental loci, heading-date data collected on the three populations indicated that major developmental loci were in other genomic locations. With no evidence of a major developmental or stress responses in this location, it is possible that this is a stress-response locus not previously identified. Chromosome 2HL has been reported as the location of cold-regulated genes *cor14b* and *blt14* (Francia et al. 2004; Cattivelli et al. 2002b). Their reported location in bin 9 is proximal to the frost tolerance QTL identified in this study (Fig. 4).

In the three populations used in this study, frost tolerance QTL identified on chromosome 5H all mapped to the same relative genomic location (Fig. 4). DGS and plant height on primary tiller QTLs also mapped to this location in the Amagi Nijo/WI2585 population (Fig. 4). Screening methods employed were able to overcome these confounding effects. This location has been implicated in numerous cold tolerance traits in other populations. These include LT50 and winter survival in the Dicktoo/Morex population (Hayes et al. 1993a; Pan et al. 1994) and survival in controlled environment and winter survival in the Nure/Tremois population (Francia et al. 2004). Choi et al. (2000) also identified two dehydrin genes (*Dhn1* and *Dhn2*) that were located in the same location as the winterhardiness QTL on chromosome 5HL in the Dicktoo/Morex population. Cold tolerance is believed to be largely due to cold acclimation that occurs when plants are exposed to cold but non-freezing temperatures (Cattivelli et al. 2002b) and is achieved at vegetative growth stages (Hayes et al. 1997). The significant QTL identified in spring × spring crosses at the reproductive stage of development indicates that the mechanism of cold tolerance may extend further than previously anticipated.

Although the frost tolerance phenotype was expressed in barley varieties with a spring growth habit, the segregation within the Japanese-derived populations suggest that (a) recessive winter allele(s) was/were potentially present at the *vrn-H1* locus. A recessive *vrn-H1* allele has been found to be the major determinant in a vernalisation-responsive phenotype and is epistatic to the *Vrn-H2* locus on chromosome 4HL (Takahashi and Yasuda 1971). Cattivelli et al. (1994) proposed that the winter growth habit is determined by an interaction between the dominant *Vrn-H2* locus and the recessive *vrn-H1* locus. It

is therefore likely that Haruna Nijo is carrying recessive alleles at the *vrn-H1* and *vrn-H2* loci, with Galleon carrying the dominant forms of these alleles. The combination of *vrn-H1* and *Vrn-H2* produces a vernalisation response (winter) phenotype, whereas all other combinations produce a spring type. This would be consistent with the segregation ratio of 3:1 spring:winter observed in the Galleon/Haruna Nijo population. Winter-hardiness has been linked to the recessive *vrn-H1* allele (Hayes et al. 1993a; Francia et al. 2004) in winter/spring crosses at the vegetative stage of development. The reproductive frost tolerance of spring lines possessing the recessive *vrn-H1* allele(s) suggests that this locus is implicated in tolerance at all stages of development.

The Franklin/Arapiles population was not segregating for winter/spring growth habit. The loci having an effect on days to heading in this population were the *denso* dwarfing locus on chromosome 3H and the *eps2* locus on chromosome 2H. Although the *vrn-H1* locus has no significant effect of days to heading, it may still have been segregating in the population, as it has a series of alleles that induce several winter to spring phenotypes (Cattivelli et al. 1994).

The group 5 chromosomes belonging to the tribe *Triticeae* have an important role in abiotic stress tolerance in a number of crops (Cattivelli et al. 2002a). The region on the long arm of the group 5 chromosomes, where the syntenous *Vrn* genes are located, have been identified as being associated with cold tolerance traits in wheat (Sutka 2001; Toth et al. 2003) and barley (Hayes et al. 1993a; Pan et al. 1994; Francia et al. 2004). These studies have predominantly focused on leaf and plant survival at the vegetative stage after an extended period of hardening. We have demonstrated that this region is also important in conferring tolerance to frost at the flowering and grain-filling stages in barley. The highly syntenous group 5 chromosomal region may now indicate that an increased tolerance to reproductive frost tolerance in wheat may also reside at this location.

Marker-assisted selection for reproductive frost tolerance

Current selection methods for reproductive frost tolerance in barley are based on field-based phenotypic observations after a frost event. This selection method can be prone to error due to within-site variation, seasonal variation, confounding effect of developmental and morphological traits and the sporadic nature of discriminating frost events. Therefore, the use of phenotypic selection in a breeding program, especially on segregating populations, is limited due to the low throughput, high risk and expense. Consequently, very few breeding programs have attempted to improve reproductive frost tolerance.

Marker-assisted selection (MAS) provides a tool for identifying individuals in a segregating population carrying favourable alleles for frost tolerance at the 2HL and 5HL loci, with high throughput and relatively low cost. MAS is especially valuable for use in a backcrossing strategy. Validation of these QTL will be achieved by integrating them into genetic backgrounds of Australian adapted genotypes. We have now commenced breeding programs in which this trait will be actively pursued via genotypic and phenotypic selection.

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