

Post head-emergence frost resistance of barley genotypes in the northern grain region of Australia

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Abstract. Post head-emergence frost causes substantial losses for Australian barley producers. Varieties with improved resistance would have a significant positive impact on Australian cropping enterprises. Five barley genotypes previously tested for reproductive frost resistance in southern Australia were tested, post head-emergence, in the northern grain region of Australia and compared with the typical northern control cultivars, Gilbert and Kaputar. All tested genotypes suffered severe damage to whole heads and stems at plant minimum temperatures less than -8°C . In 2003, 2004 and 2005, frost events reaching a plant minimum temperature of $\sim -6.5^{\circ}\text{C}$ did not result in the complete loss of grain yield. Rather, partial seed set was observed. The control genotype, Gilbert, exhibited seed set that was greater than or equal to that of any genotype in each year, as did Kaputar when tested in 2005. Thus, Gilbert and Kaputar were at least as resistant as any tested genotype. This contrasts with trial results from the southern grain region where Gilbert was reported to be less resistant than Franklin, Amagi Nijo and Haruna Nijo. Hence, rankings for post head-emergence frost damage in the northern grain region differ from those previously reported. These results indicate that Franklin, Amagi Nijo and Haruna Nijo are not likely to provide useful sources of frost resistance or markers to develop improved varieties for the northern grain region of Australia.

Additional keywords: in-head frost, reproductive frost, spring radiant frost.

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Introduction

Post head-emergence frosts are a serious limitation in the northern grain region of Australia, causing yield reductions due to (i) direct frost damage, and (ii) late planting to minimise frost risk. Yield losses due to irregular frosting of barley are a significant cost to producers. This occurs despite growers minimising the risk of frost damage by planting later or using longer season varieties. Frost minimisation strategies that delay crop flowering increase the likelihood of stress during the grain-filling period due to the depletion of stored soil moisture combined with rapidly rising temperatures late in the season. This hot and dry finish dramatically reduces yield potential, resulting in yield losses that are difficult to quantify but are thought to exceed losses due to direct frost damage. In the northern grain region of Australia, dramatic increases in yield in the order of 30–50% are observed when early flowering cereal crops escape frost. Yield declines of as much as 16% for each week that anthesis is delayed past the optimum time have been reported (McDonald *et al.* 1983; Woodruff and Tonks 1983). A compromise between the effects of frost and drought

must be reached to minimise the loss of yield potential in the face of frost risk (Woodruff and Tonks 1983).

Although many elite winter habit cereals, in vegetative stages, show significant tolerance to cold (Fowler and Carles 1979), both winter and spring types suffer severe damage at more moderate temperatures during the reproductive stages (Fuller *et al.* 2007). Post head-emergence frost damage to winter habit cereals has been periodically reported over many years (Livingston and Swinbank 1950; Chatters and Schlehuber 1953; Paulsen and Heyne 1983). Evidence suggests that winter habit cereals exhibit similar levels of post head-emergence frost susceptibility to those of spring habit genotypes despite a higher level of resistance in the vegetative stages (Livingston and Swinbank 1950; Fuller *et al.* 2007).

In winter habit wheat, the transition from the vegetative to reproductive growth stages is pivotal to the regulation of low-temperature tolerance (Mahfoozi *et al.* 2001a). In the northern cropping region of Australia, major economic losses due to vegetative frosting are rare (Single 1991). Freezing tolerance in the vegetative stages has been the focus of rigorous

investigation (Fowler and Carles 1979; Fowler 2008). In contrast, research effort into frost during stem elongation, booting and post head-emergence has been more modest. Of the frost studies during later developmental stages, most work focussed on wheat (Single 1964, 1984, 1988) and indicated that frost susceptibility generally increased with plant maturity. In particular, wheat plants became more susceptible during spike emergence from the boot (Livingston and Swinbank 1950; Single 1964; Afanasev 1966; Paulsen and Heyne 1983). In Australia, severe damage to crops before heading is not common, and when it does occur there is typically time for later-formed tillers to partially compensate, enabling reasonable recovery of yield (Single 1991).

A framework similar to that outlined by Levitt (1980) for drought has been useful to describe post head-emergence frost resistance in wheat (Frederiks *et al.* 2008). Using this framework in barley, post head-emergence frost adaptation can be discussed in terms of frost escape and resistance. For example, frost escape can be viewed as the evasion of frost by differences in phenology (i.e. floral structures do not face the challenge of frost). Alternatively, resistance is achieved when plants face the challenge of frost (i.e. floral structures are exposed to frost) and exhibit either some level of freezing adaptation (tolerance) or avoidance mechanisms such as supercooling. This study focused on discerning differences in frost resistance which could be due to tolerance and/or avoidance mechanisms.

Increasing post head-emergence frost resistance of Australian cereals is an important objective and has been under investigation for more than a century (Farrer 1900). Yield gains have been made through better management of frost risk using frost escape, particularly by optimising the combination of planting dates and varieties (Woodruff 1992; Cahill *et al.* 1995). However, there has been little progress in identifying true genetic post head-emergence frost resistance. The difficulties inherent in frost research have meant relatively few research papers have been published. Encouraging results have been reported periodically (Fletcher 1983; Maes *et al.* 2001; Reinheimer *et al.* 2004; Fuller *et al.* 2007). However, to date, no successful variety with useful levels of resistance has been developed (Fuller *et al.* 2007). Lines with putative post head-emergence frost resistance have been identified opportunistically from frosted breeding trials. However, in some cases, small differences in phenology may affect the relative susceptibility of the head and floral structures of a particular line exposed to any individual frost event. Additionally, temperature differentials within a trial due to spatial variation in the crop canopy may vary the intensity of the frost exposure for particular lines (Frederiks *et al.* 2004a). The position within the canopy can dramatically affect the temperature recorded (Marcellos and Single 1975). The density of the canopy can also influence the damage observed (Woodruff *et al.* 1997). In order to improve the efficiency of research and breeding, a rigorous testing method that minimises the confounding effects of small differences in phenology and spatial differences in temperature is required to assess material.

There have been reports of barley lines, including Haruna Nijo and Amagi Nijo, exhibiting superior reproductive frost adaptation compared with current cultivars in the southern grain region of Australia (Reinheimer *et al.* 2003, 2004). Quantitative trait loci (QTL) were reported on chromosomes

2HL and 5HL (Reinheimer *et al.* 2004). Fine molecular mapping of these regions and studies of developmental and morphological traits associated with the reproductive adaptation in Haruna Nijo and Amagi Nijo have also been reported (Chen *et al.* 2009a, 2009b, 2009c).

In this study, the post head-emergence frost resistance was assessed in barley lines reported to show improved reproductive frost adaptation (Reinheimer *et al.* 2004). The aim was to determine whether any such differences are large enough to offer a useful level of resistance when tested in the northern Australian grain region. The lines were compared with two typical northern control varieties, Gilbert and Kaputar, using a screening method developed to allow post head-emergence frost resistance to be accurately assessed in isolation from frost escape mechanisms.

Materials and methods

Plant material

Five barley genotypes (*Hordeum vulgare* L.) that have been previously tested in the southern Australian grain region were assessed. Seed of these varieties was sourced from the University of Adelaide. Three of these genotypes are elite varieties in southern Australia (Franklin) or Japan (Amagi Nijo and Haruna Nijo) and have been used as resistant parents to develop breeding and mapping populations in southern Australia. The other two genotypes were Ethiopian accessions Icarda #81 and Icarda #70, which ranked in southern Australia as resistant and susceptible, respectively (Reinheimer *et al.* 2003, 2004). These lines were compared with the northern Australian cultivars, Gilbert and Kaputar. From previous work, these varieties are considered representative of current northern cultivars. For this reason they are described as controls representing a baseline or *status quo* level of resistance upon which we would aim to improve. Seed of northern control varieties was obtained from the Northern Barley Improvement Program at the Queensland Government's Hermitage Research Facility, near Warwick, Queensland. All tested genotypes were of spring habit.

Trial sites

Field trials were established at two sites: the DEEDI farm site at Kingsthorpe (west of Toowoomba, 27.51°S, 151.78°E, ~440 m a.s.l.) and at Hermitage Research Facility (near Warwick, 28.21°S, 152.10°E, ~480 m a.s.l.). These sites have been shown to consistently experience damaging radiant frosts between May and August, and to vary in minimum temperatures on a given night, exposing plants to a wide range of frost intensities over a typical season.

Trial design

In 2003, 2004 and 2005, Gilbert, Icarda #81, Icarda #70, Franklin, Amagi Nijo, Haruna Nijo, as well as Kaputar in 2005 only, were planted at both the Kingsthorpe and Hermitage sites. Genotypes were established in a randomised design at each planting date with two or three sowing dates at each site. Trials were fully irrigated and fertilised to provide non-limiting levels of moisture and nutrients. Seed was hand-sown in 5-m rows with row spacing of 0.3 m. The planting dates at each site were chosen to ensure

flowering throughout the peak frost risk period from late May to the end of August. The first sowing was established as early in April as practical. Subsequent plantings were established at ~3-weekly intervals. At the end of each row, supplemental artificial lighting was provided by 100-W incandescent bulbs or 18-W compact fluorescent bulbs at 0.8-m spacing and ~1 m height above ground level to increase daylength to 18 h in the vicinity of the lights. A 1-m wheat buffer strip was planted directly below the lights with test rows starting 0.5 m from, and running 5 m perpendicular to the line of lights. Light intensity diminished with distance along the length of each test row creating a gradient in the daylength extension effect. A wide spread of flowering times was generated for each genotype by the serial plantings in combination with the gradient in daylength extension along the test rows. Heads of similar developmental stage were selected among genotypes of different phenology by selecting heads at various distances from the lights. This spread in flowering time allowed comparisons between genotypes of varying phenology to be made at a similar developmental stage during each natural frost event. Genotypes were compared within individual sowing times unless otherwise stated. A relatively uniform, closed canopy was developed by modifying seeding rates (dependent on germination rate) to provide a target population density of ~130 plants m⁻². Guard rows were planted around the test rows to form a standardised canopy to the edges of the trial.

Temperature measurements

Plant minimum temperatures at the top of the canopy were measured using fine probes (1 × 8 mm PB-4711 Hastings Data Loggers, Port Macquarie, NSW, Australia) and logged using 'Tinytag Plus' data loggers recording minimum temperature at 5-min intervals (Gemini Data Loggers, Chichester, West Sussex, UK). Temperatures reported are subject to the internal accuracy of the loggers and probe, quoted as ±0.2°C from 0 to 70°C and ±0.3°C at -20°C (PB-5006 Hastings Data Loggers), respectively. Vertical temperature variation within the canopy can be in the order of several degrees. How and where temperatures are measured in the canopy can affect the values observed (Marcellos and Single 1975). Plant minimum temperatures were recorded using probes attached with adhesive tape to the leaf blade of the upper most expanded leaf, unscreened and exposed to the night sky. These temperatures are the minimum value observed during each 5-min interval, not averages. The complex association between crop, air and Stevenson screen temperatures is discussed in the *frost characterisation* section later in this paper.

Assessing material and data analysis

Individual heads were tagged on the day of a frost event, ensuring that only heads known to have been exposed to a particular frost are assessed for damage. This can be particularly important during the period of head-emergence, as heads that have escaped frost damage in the boot during the night may emerge the next day. Material from each line was marked at a uniform stage of head-emergence from the boot. The stage of flowering of the marked material may vary among test lines due to differences in the timing of anthesis relative to head-emergence between barley genotypes. Importantly, every effort was made to ensure

that previously frost damaged material was not marked. This screening method allowed post head-emergence frost resistance to be accurately assessed with minimum influence from frost escape mechanisms. Symptoms of damage were allowed to develop in the field. Tagged heads were assessed within 14 days of the frost event for stem and floret damage. Material was assessed before forecast frosts so as to avoid confounding effects from additional frost damage. Where heads survived, but individual florets within each head were damaged, the number of potentially viable florets and the total number of grains forming were recorded. The proportion of grain set per spike was calculated using the formula: undamaged grain number/total number of potentially viable florets. The number of heads available and assessed varied from 10 to >45 per genotype with the exception of Franklin for which 3–12 heads per event were assessed.

During the third frost event in 2004, observations were made across two planting dates. For this analysis, time of sowing 2 and time of sowing 3 were used as the blocking factors in a randomised block design, with varying numbers of frosted heads assessed for each field plot. A linear mixed model was fitted to the data with terms for genotype and time of sowing. Heads within plots formed the residual term in the model. Variance components were estimated using the residual maximum likelihood method (REML; Patterson and Thompson 1971) and best linear unbiased estimates (BLUEs) and their standard errors were produced for the fixed effect of genotype. The analysis was implemented in GenStat (Payne *et al.* 2008). All other observations are presented for plants from one planting date only. For this data, the mean proportion of grain set was calculated for all heads counted for each genotype at each event, and the 95% confidence interval for the mean was calculated using a binomial distribution.

Results

Frost frequency and intensity

Frosts at plant minimum temperatures between ~-6 and -8°C often resulted in damage to individual barley florets but did not usually damage the entire head nor cause serious damage to the stem. Frosts colder than ~-8°C typically killed all emerged spikes, often severely damaging the supporting stem. Severe frost can also damage heads entirely contained within the leaf sheath.

Daytime maximum temperatures were typically observed soon after noon. The most rapid temperature drops were often observed in the late afternoon and early evening. Plant minimum temperatures were observed at, or just before, dawn. The absolute plant minimum temperatures reported in this study were only transient. Plant temperatures increased rapidly after dawn. Mid-winter daytime plant temperatures were typically over 15°C. Plants exposed to direct sunlight could reach temperatures over 30°C. This means that even in mid winter, plant temperatures could change by more than 35°C during the course of the day, from the pre-dawn minima to the daytime maxima less than 7 h later. The test sites experienced several crop damaging frosts each season. Frost events often occurred over successive mornings. Frosts were observed sporadically from late May until September. For example, during the peak

frost risk period of 2004 at the Hermitage site there were at least eight frost events where plant temperatures dropped below -6°C with potential to cause serious damage to barley. A frost of -11.5°C at canopy height on 30 June 2004 caused severe stem and head damage to all emerged and booting spikes, resulting in a period when there were few, if any, undamaged spikes for testing in the three subsequent frosts on 7, 9 and 19 July when plant temperatures dropped below -6°C .

Post head-emergence frost damage

In 2004 and 2005, useful quantitative data was obtained from four frost events with similar plant minimum temperatures, in the order of -6.5°C , at the Hermitage Research Facility trial site. The proportion of grain set in the tested barley lines is presented in Figs 1–4 (presented chronologically). For the third frost event (13 September 2004), plants from two planting dates at a similar stage of development were available for measurement (Fig. 3*a, b*). The analysis of proportional grain set from the frost on 13 September showed planting date did not have a significant effect; however, the effect of genotype was highly significant. Gilbert exhibited a significantly higher grain set than any other tested genotypes (Table 1). Icarda #81, Icarda #70 and Haruna Nijo all exhibited lower proportional grain set with predicted means significantly below that of Gilbert. The predicted mean was lower again for Amagi Nijo and Franklin, but not significantly lower than Haruna Nijo (Table 1). Time of sowing did not show a significant effect in this instance, however differences in the temperature

experienced and damage between plants from different sowing dates have previously been reported (Frederiks *et al.* 2004*a*). Sowing time was used as a blocking factor in this case as the overall level of grain set and the ranking of genotypes were similar for the two times of sowing (Fig. 3*a, b*). No significant interaction between genotype and time of sowing was found in this analysis.

Data from two earlier frost events in 2004 (Figs 1 and 2) and the frost in 2005 (Fig. 4) support the result from the third frost event in 2004 (Fig. 3*a, b*). Grain set differed between barley lines. In 2004, no tested line consistently performed better than the control Gilbert in any of the three frost events. In fact, Gilbert retained a higher or similar grain set when compared with any of the tested genotypes. Franklin, due to differing phenology, was only able to be measured during two frosts in 2004 (5 and 12 heads, respectively), when it ranked with Icarda #70, Haruna Nijo and Amagi Nijo, which had low rates of seed set (Fig. 3*a, b*). These results were confirmed in 2005 with a frost reaching a plant minimum temperature of -6.5°C on 9 August (Fig. 4). This event demonstrated that a second typical northern control, Kaputar, groups with Gilbert. Both control lines showed a higher proportion of grain set than other test genotypes (Fig. 4). For a frost plant minimum temperature of -8.1°C on 17 June 2003 at Kingsthorpe, grain set of all tested types was low with proportion of grain set less than 0.05 for each genotype (data not presented). During this more severe event, no tested barley line showed a potentially useful increase in grain set compared with the control genotype, Gilbert. Therefore, following six frost events over 3 years, no test line displayed a

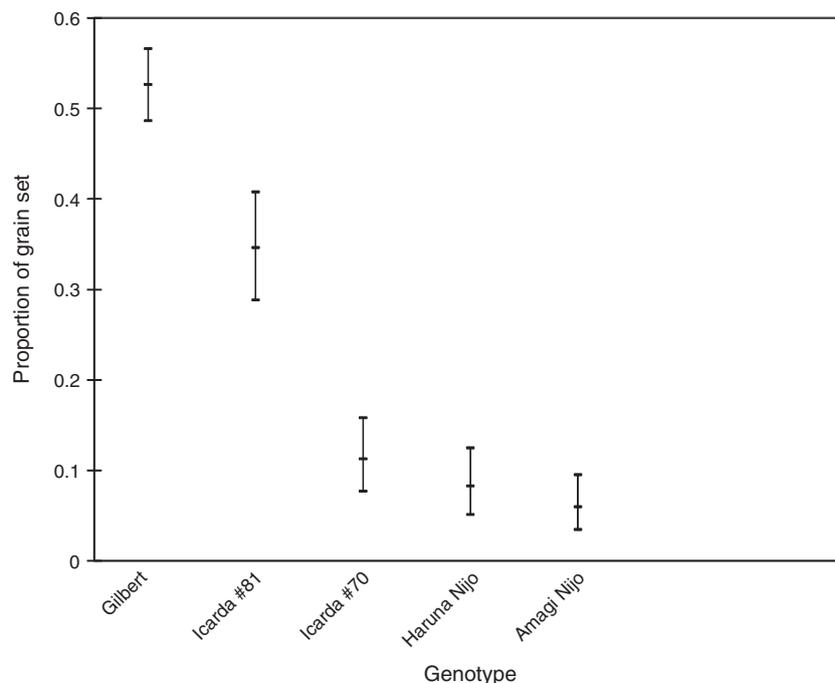


Fig. 1. Proportion of grain set for test barley genotypes Amagi Nijo, Haruna Nijo, Icarda #70 and Icarda #81 compared with the northern frost control Gilbert, exposed to a frost of -6.5°C plant minimum temperature at canopy height (Stevenson screen temperature -4°C) at Hermitage Research Facility on 18 June 2004. Values represent the mean proportion of grain set per spike. Error bars represent the 95% confidence interval calculated for a binomial distribution.

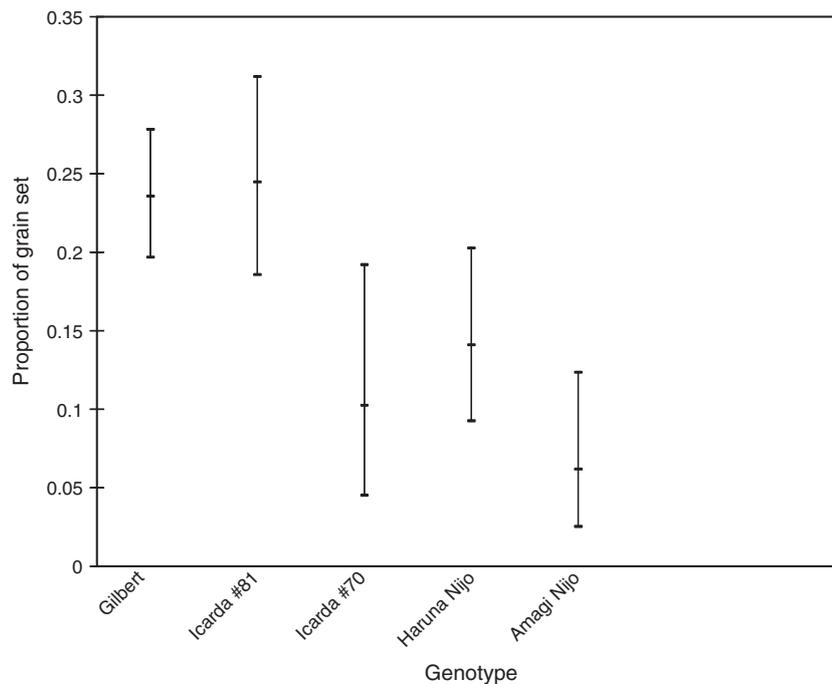


Fig. 2. Proportion of grain set for test barley genotypes Amagi Nijo, Haruna Nijo, Icarda #70 and Icarda #81 compared with the northern frost control Gilbert, exposed to a frost of -7.0°C plant minimum temperature at canopy height (Stevenson screen temperature -3.4°C) at Hermitage Research Facility on 13 August 2004. Values represent the mean proportion of grain set per spike. Error bars represent the 95% confidence interval calculated for a binomial distribution.

level of reproductive frost adaptation that would offer an advantage over the existing cultivar Gilbert (and Kaputar when tested in 2005).

Discussion

Frost characterisation

It is difficult to adequately describe a natural field frost using a single temperature measure. The more sophisticated the equipment used and the more detailed the measurements made to characterise a frost event, often the more complex the phenomenon appears (Single 1991). It is important to consider how the severity of frost damage and the temperatures observed in this study might relate to those recorded in Bureau of Meteorology stations, experienced in grower's crops, and to those reported in other studies from southern Australia (Reinheimer *et al.* 2003, 2004). There are difficulties in applying plant minimum temperatures, as reported in this study, to grain grower's paddocks. Measuring plant minimum temperature using fine, fragile thermistor probes is difficult in commercial crops. These probes need to be regularly repositioned within the canopy as the crop develops and whenever they become dislodged. Stevenson screen temperatures are typically several degrees milder than plant minimum temperatures measured in this manner. However, this difference between screen and plant temperatures may vary from one frost event to another. The screen temperatures at the Hermitage Research Facility were typically $2\text{--}4^{\circ}\text{C}$ milder than the plant minimum temperature for the frosts reported in this study. From a practical

perspective, the closest Bureau of Meteorology Station may be some distance from an individual commercial crop and experience significantly different minimum temperatures. For grain growers, a more useful measure is minimum air temperature at crop head height (as described in Woodruff *et al.* 1997). Minimum air temperatures measured during damaging frosts are typically at least $1\text{--}2^{\circ}\text{C}$ milder than plant minimum temperature measured in the manner described here. Unlike Stevenson screen temperatures, minimum air temperatures within the crop more closely follow plant minimum temperatures. The reduced grain set observed in this study would be expected to result in moderate losses in commercial crops, suggesting that the plant minimum temperatures ($\sim -6^{\circ}\text{C}$) reported in this study are of a temperature range relevant to commercial barley crops in the northern region.

Screening for resistance

Serial planting generated post head-emergence plant material throughout the peak mid-winter frost period. Photoperiod extension resulted in a spread in phenology along each test row. Assessing differential floret damage required newly emerged and undamaged spikes to be available when natural frosts occurred. The availability of undamaged material limited the number of frosts from which data could be generated. At the two trial sites, with multiple plantings over three seasons, six frost events were observed where suitable previously undamaged plant material of several genotypes was available during frosts of a temperature range that produced differential damage.

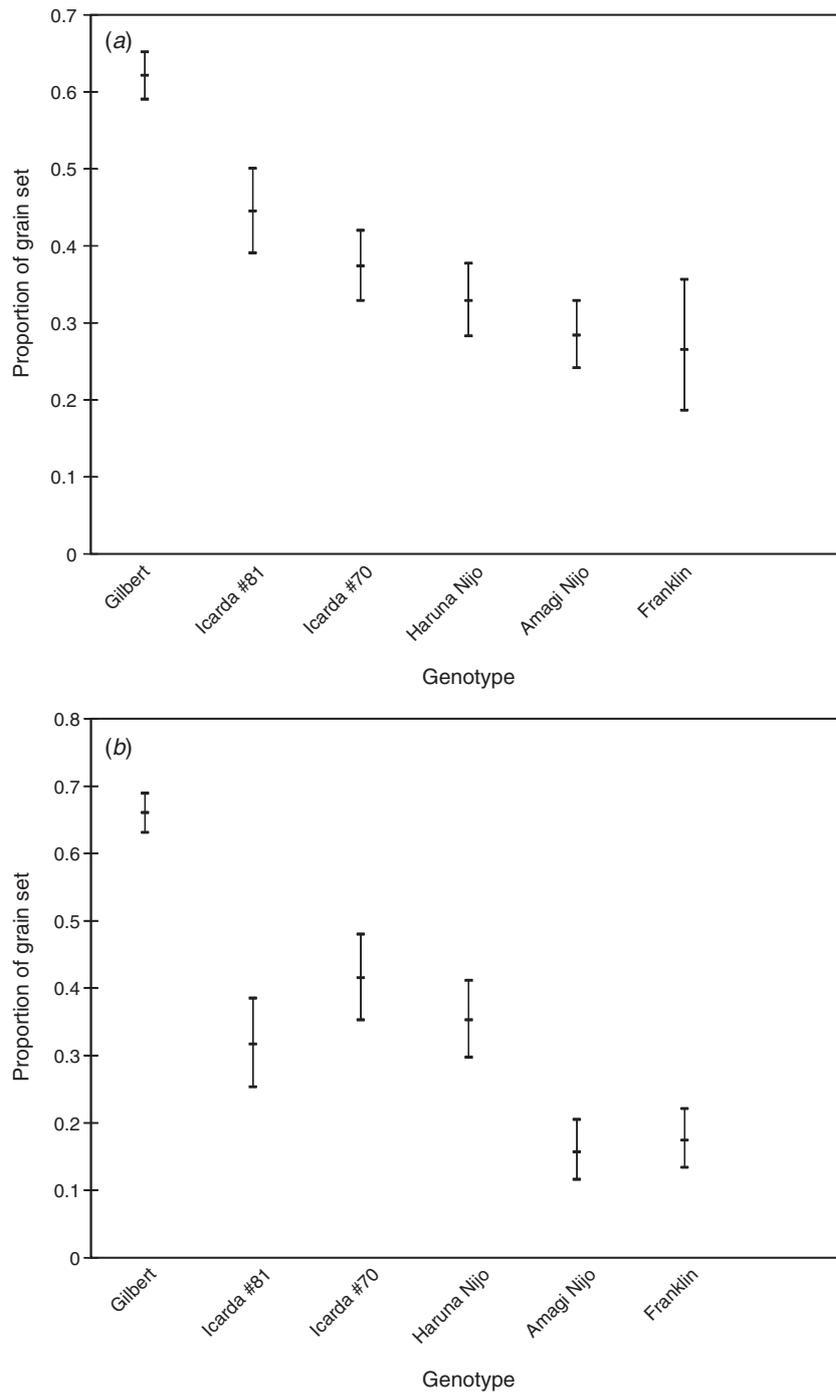


Fig. 3. (a) Proportion of grain set for test barley genotypes Amagi Nijo, Haruna Nijo, Icarda #70, Icarda #81 and Franklin compared with the northern frost control Gilbert, exposed to a frost of $\sim -6.5^{\circ}\text{C}$ plant minimum temperature at canopy height (Stevenson screen temperature -2.3°C) at the Hermitage Research Facility on 13 September 2004 (sowing time 3 with photoperiod extension). Values represent the mean proportion of grain set per spike. Error bars represent the 95% confidence interval calculated for a binomial distribution. (b) Proportion of grain set for test barley genotypes Amagi Nijo, Haruna Nijo, Icarda #70, Icarda #81 and Franklin compared with the northern frost control Gilbert. As in Fig. 3a exposed to a frost on 13 September 2004 at the Hermitage Research Facility, but plant material from sowing time 2 with a lesser photoperiod extension effect. Values represent the mean proportion of grains set per spike. Error bars represent the 95% confidence interval calculated for a binomial distribution.

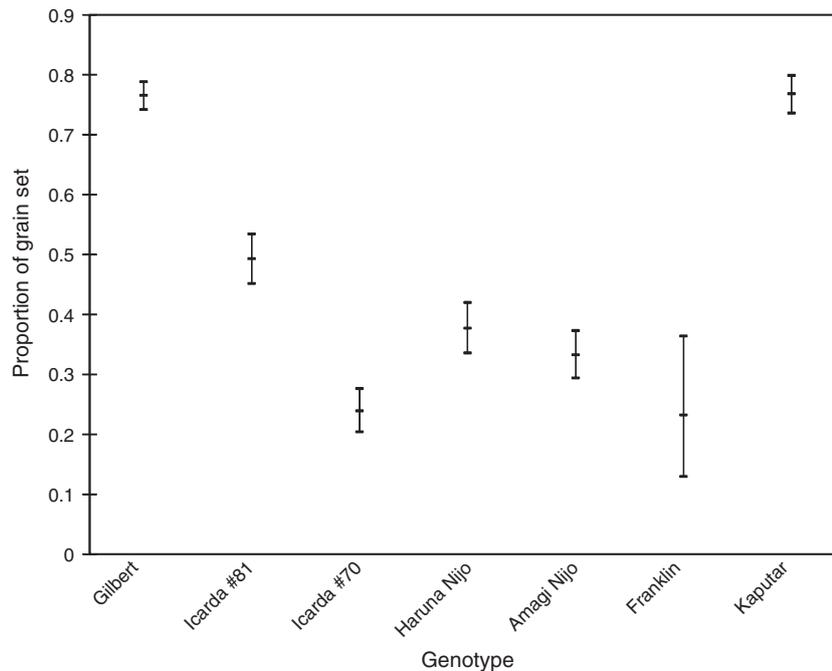


Fig. 4. Proportion of grain set for test barley genotypes Amagi Nijo, Haruna Nijo, Icarda #70, Icarda #81 and Franklin compared with the northern frost controls Gilbert and Kaputar, exposed to a frost event of -6.5°C plant minimum temperature (Stevenson screen temperature -3.4°C) at the Hermitage Research Facility on 9 August 2005. Values represent the mean proportion of grain set per spike. Error bars represent the 95% confidence interval calculated for a binomial distribution.

Table 1. Predicted mean proportional grain set for six barley varieties exposed to frost at Hermitage Research Facility on 13 September 2004 when crop canopy temperature dropped to -6.5°C (Stevenson screen -2.3°C)

Values represent the predicted means from two times of sowing for Frost 3 as represented in Fig. 3a and b. Values assigned the same letter in the 'Significance' column are not significantly different ($P > 0.05$)

Variety	Predicted mean grain set	Significance ($P < 0.05$)
Gilbert	0.64	a
Icarda #81	0.38	b
Icarda #70	0.38	b
Haruna Nijo	0.32	bc
Amagi Nijo	0.22	c
Franklin	0.21	c

Trials characterising only a small number of lines were carefully established to minimise canopy variation. Plant minimum temperatures were logged. Individual heads were marked within hours of well characterised frost events. This robust method allowed post head-emergence frost resistance to be assessed during naturally occurring frost events, while minimising the confounding effects of frost escape due to developmental differences in the emergence of heads from the boot. However, some caution needs to be exercised when interpreting this data. Genotypes were planted in single rows for each planting time at each site. The proportional grain set was derived from various numbers of individual heads available to be sampled within these rows. Despite this limitation, the results

from all five events that resulted in differential damage at two sites over three seasons showed similar genotype rankings. Franklin, Haruna Nijo, Amagi Nijo, Icarda #70 and Icarda #81 exhibited no advantage, post head-emergence, over the northern control variety Gilbert (nor Kaputar when tested in 2005). Thus, the plant damage results we report contrast with previous reports from southern Australia where Franklin, Haruna Nijo and Amagi Nijo were ranked as more frost resistant compared with Gilbert (Reinheimer *et al.* 2003, 2004).

Results differ between regions

It is possible that the nature of frost events for which the data was collected may differ between the southern and northern grain regions. Potentially, this could have contributed to the difference in genotype rankings. The Kingsthorpe and Hermitage sites routinely experience multiple frosts annually in a range between -5 and -8°C plant minimum temperatures where differences in grain set can be observed. Temperatures reported by Reinheimer *et al.* (2003) (using thermocouples at a height of 0.8 m) may be less severe than the plant minimum temperatures reported here. Even though the reported minimum temperatures for frosts are different between regions, similar ranges of proportional grain set, or the inverse parameter of 'frost-induced sterility' are reported (Reinheimer *et al.* 2003, 2004). The similarity in the range of proportional grain set between the two regions indicates that, in terms of plant response, the severity of the frost events was similar between regions. Thus, differences in the ranking of genotypes were

observed even though the ranges of absolute damage levels were similar for the two regions.

The change in the ranking of genotypes between the southern and northern grain regions would initially appear to be a typical case of genotype by environment interaction ($G \times E$) where the ranking of genotypes changes from one environment to another (reviewed in Cooper *et al.* 1995). However, as in studies from South Australia, ICARDA #81 exhibits higher proportional grain set than ICARDA #70 in 4 of the 5 frost treatments in 2004 and 2005 (Figs 1, 2, 3a and 4). This similar relative ranking of ICARDA #81 and ICARDA #70 indicates that some genotypes, at least, do rank similarly in the northern and southern grain regions of Australia. To be confident that regional differences in genotypic ranking arise from the interaction between genotype and environment, it is important that the data on genotypes be collected in a similar manner for each environment. However, the potentially confounding effect of differences in methodology between regions currently makes it difficult to be confident that $G \times E$ is the major contributor to differences in proportional grain set rankings. Hence, some discussion on the differences between the trial methodologies employed in each region is warranted.

The northern studies involved only a small number of lines hand-planted to carefully control plant density, resulting in a continuous plant canopy, with the aim of minimising spatial temperature variation within plots. Daylength extension was used to bring lines to a common stage of head-emergence for testing. Trials in the south had multiple plantings but did not employ daylength extension. Comparing genotypes of different phenology at similar developmental stages is inherently difficult. Comparing genotypes across planting times also presents difficulties. Small differences, for example canopy differences between plots, can affect the temperature of frosts experienced (Marcellos and Single 1975; Woodruff *et al.* 1997; Frederiks *et al.* 2004a). Small differences in temperature experienced during frosts can have relatively large effects on plant damage (Frederiks *et al.* 2004a). When comparing genotypes exhibiting a quicker phenology with slower maturing types, by definition the quicker maturing genotypes will be in later planting time plots than genotypes with a slower phenology. This may introduce a reproducible confounding effect of planting date on any genotypic comparisons. Such a confounding effect may not be able to be removed using statistical techniques. In contrast, photoperiod extension allows genotypes of differing phenology to be compared within a single time of sowing during the same frost event at a similar growth stage, thus reducing the potential for differences in frost damage between times of sowing. Differences in damage between times of sowing may (Frederiks *et al.* 2004a) or may not be observed (13 September 2004, Fig. 3a, b). When using photoperiod to bring genotypes of different phenology to the same growth stage, we must be alert to any potential for differences in photoperiod affecting frost damage. However, the data does not support this assertion. For example, during the frost of 13 September 2004, plants of similar developmental stage were marked from both a late and earlier time of sowing by selecting spikes close to, or remote from, the lights, respectively (Fig. 3a, b). Genotypic rankings for the two times of sowing are similar (Fig. 3a versus b). In previous studies photoperiod extension has been linked to a

loss in vegetative cold hardiness in winter cereals (Mahfoozi *et al.* 2001a, 2001b). However, the authors proposed that it was the stage of development modified by photoperiod and vernalisation which resulted in a loss of vegetative cold hardiness, rather than cold hardiness being altered by photoperiod *per se*. In this study the effect of photoperiod is being used to reduce differences in the developmental stage between test genotypes.

In addition to trial design, the developmental stage of material tested differed between regions. In the present study, all assessed material had, at least partially, emerged from the boot at the time of the frost and was without symptoms of previous damage. To assess frost-induced sterility after frost events in South Australia, material was marked just before anthesis (Reinheimer *et al.* 2004). In barley, anthesis can occur before awn emergence and is not as easily detectable as in some other cereals (Zadoks *et al.* 1974). There is good evidence in wheat that the breaking of the boot is critical for heads to become fully susceptible to spring radiant frost (Livingston and Swinbank 1950; Single 1964; Afanasev 1966; Paulsen and Heyne 1983). Similar information for the relative susceptibility of barley pre- or post-emergence has not yet been published. However, recent studies in northern Australia indicate that barley, like wheat, increases in susceptibility as heads emerge from the boot (Frederiks *et al.* 2011). Therefore, it is likely that differences in head-emergence at anthesis could alter the susceptibility and damage scores of barley lines tested at, or before, anthesis.

Applicability of tested lines to barley improvement

Reinheimer *et al.* (2004) associated frost sensitivity of reproductive tissues in Amagi Nijo \times WI2585 and Haruna Nijo \times Galleon populations with QTL located on chromosomes 2H and 5H. Chen *et al.* (2009c) reported that frost adaptation was associated with shorter spikes, due to reduced floret number (5HL) and shorter rachis internodes (2HL). If like wheat, the susceptibility of these barley lines increases as the heads emerge from the boot, the compact spike may delay head-emergence and similarly full susceptibility. If so, this frost adaptation mechanism is a form of frost escape. Such an escape strategy may only be effective for a short time before head-emergence and therefore have little effect on decreasing the overall frost risk in commercial crops. Any commercial advantages of compact spikes over other frost escape strategies, such as longer phenology, has yet to be demonstrated. Further, any frost advantage of a compact spike would need to be assessed against any potential yield losses due to reduced spike size.

Artificial freezing chamber screening

Chen *et al.* (2009b) describe a method using an artificial freezing chamber to characterise the 2H QTL from Amagi Nijo. Plants were tested at booting (Zadoks 49) through to the first spikelet becoming visible (Zadoks 50) or half inflorescences emerged (Zadoks 54) using an electrolyte conductivity assay (Chen *et al.* 2009b). As discussed above, differences in the exposure of the floral tissues to frost may result in differences in susceptibility, resulting in reproducible varietal differences in frost damage scores due to phenology rather than frost resistance (as defined in the introduction; Frederiks *et al.* 2008). In the present study, Amagi Nijo showed no improved post head-emergence frost

resistance over control varieties, suggesting limited application for the Chen *et al.* (2009b) screening method for winter cereal improvement, at least in northern Australia.

A particular problem of freezing chamber studies is the apparently random occurrence of supercooling escapes. For example, infrared thermography studies in the Australian Genome Research Facility freezing chamber (Fuller *et al.* 2006) would suggest a large proportion of plants supercooled, escaping ice formation. The efficiency of freezing can be improved with the application of water and a nucleator such as the commercial *Pseudomonas* bacterial extract Snomax (Johnson Controls, Inc., Milwaukee, WI, USA) (Chen *et al.* 2009b). Similar results have been observed using applied water and ice nucleation via CO₂ spray (Single 1991). However, supercooling can still be observed in plant tissues with applied Snomax (M. Fuller, J. Vickers, T. Frederiks, unpubl. data). The Single and Marcellos method offers the advantage, over ice nucleating bacteria or Snomax, of being able to 'nucleate' the applied water with the CO₂ spray at a temperature and/or time of the researcher's choice. Reproducibility of results is a problem when assessing material in freezing chambers (Single 1991; Frederiks *et al.* 2004b). From the results published (Chen *et al.* 2009b), it is not possible to determine whether the Chen method offers any improvement in reproducibility of results.

Conclusions

No tested barley line showed resistance to frosts below $\sim -8^{\circ}\text{C}$ minimum plant temperature at canopy height. Frosts of this severity caused damage to whole spikes and stems. Thus, none of the lines would be considered resistant to frost causing the most serious crop damage to commercial cereal crops.

For frost events between ~ -6 and -8°C , differences between genotypes were observed in the proportion of grains set. However, the ranking of varieties for proportional grain set in trials in the northern grain region differed markedly from tests in the southern grain region. Although the genotypes Haruna Nijo, Amagi Nijo and Franklin have been used as 'resistant' parents and to identify molecular markers for frost adaptation in southern Australia (Reinheimer *et al.* 2004), they did not exhibit superior post head-emergence frost resistance when tested in the northern grain region. In fact, the converse was true. The results of this study suggest that frost adaptation derived from these genotypes are unlikely to provide reliable sources of post head-emergence frost resistance in commercial varieties or of molecular markers useful for barley improvement in northern Australia.

It is currently not possible to determine whether the difference in rankings between regions is largely determined by $G \times E$ or whether methodological differences have contributed. Collaborative research in the southern, western and northern Australian grain regions is currently underway to further explore these differences. The developmental stage of the plant material assessed was an important difference between this study and others previously reported (Chen *et al.* 2009a, 2009b, 2009c). The relative susceptibility of barley before or after head-emergence from the boot may be important. To maximise research and breeding gains, screening methodologies that minimise frost escape should be used. The problem of spring radiant frost needs to be better defined and a

more descriptive framework (Frederiks *et al.* 2008) used in future discussions of frost adaptation.

Field-screening a wide range of germplasm for frost resistance should remain a high priority, particularly in the light of current uncertainty about the usefulness of artificial screening methods. Due to the intensive nature of field screening, so far, only a relatively small number of lines have been assessed. As more diverse genetic material is screened, and if the interactions of phenology and frost sensitivity are fully accounted for, the potential exists to identify barley genotypes with post head-emergence frost resistance.

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