



## **Genetic variation for spring radiation frost tolerance in barley**

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## **Abstract**

Spring radiation frost can cause significant losses in productivity and a reduction in malting quality of barley throughout Australian barley growing regions. Frost damage in barley has been prevalent in recent years prompting a GRDC funded project to identify spring radiation frost tolerance in barley.

The project aims to characterise genetic variation for tolerance to spring radiation frost in barley through field-based screening. If genetic variation is found, the development of a mapping population to identify molecular markers linked to genes/QTLs conferring frost tolerance in barley will commence. The impact of frost damage on malting quality is also under investigation.

Germplasm from areas throughout the world where spring frost occurs have been selected for screening, as well as commercial winter and spring varieties, selected landraces and mutant gene introgressions. Screening is being carried out at three locations in SA where spring radiation frost is frequent. The evaluation of frost damage includes traits such as electrolyte leakage; tiller, spike and floret mortality; pinched grain and grain weight.

This project will lay the foundations for future barley breeding programs to develop varieties with improved tolerance/avoidance to spring radiation frost and provide an increased understanding of the effects of spring radiation frost on key malting quality parameters.

## **Introduction**

Spring radiation frost causes direct and indirect losses in grain yield and quality in barley. Direct losses include tiller, spike and floret mortality and pinched grain. Indirect losses result from the need to delay sowing to avoid frost, reducing yield due to grain set and grain filling in sub-optimal conditions. It has been estimated that in Victoria and South Australia, the annual costs of frost are \$9.2m from direct yield losses, \$22.5m from indirect yield losses and \$1.9m from quality downgrading

(Jefferies pers. comm.). As frost is a very random and sporadic event, this burden is unevenly spread over the barley growing area and often a relatively small number of farmers suffer very high economic loss.

While there has been much research carried out into freezing tolerance during vegetative growth of barley, especially in the northern hemisphere, no comprehensive study has been undertaken to determine whether there is genetic variation for spring radiation frost tolerance from head emergence through to late grain fill. This is the period where barley is most susceptible and where the most significant yield loss is encountered in Australia. Genetic variation is most likely to exist in landraces or wild barleys that experience frequent exposure to sub-zero temperatures at late stages of crop development.

The effects of low temperatures during grain filling on specific malting quality traits are largely unknown. The receival standards for frost damage are based on visual assessment on delivery, but the relationship between visual assessment and malting quality has not been critically assessed.

This project will determine if genetic variation for spring radiation frost tolerance exists within a diverse range of barley germplasm. If genetic variation for frost tolerance is found then mapping of genes/QTLs conferring frost tolerance will commence using mapping populations of selected parents. By providing a better understanding of the impact of frost on malt quality, the project will also provide an improved understanding of the effects of spring radiation frost on key malt quality parameters with the potential for revised receival standards.

## Materials and Methods

104 genotypes are undergoing testing using field based screening techniques. To provide the greatest potential for identifying genetic variation for frost tolerance a wide range of germplasm was collected. Winter barley with frost tolerance during early growth stages was selected for screening to examine whether any mechanisms of tolerance at the vegetative stage correlated with responses after head emergence. Landraces from areas of the world where radiation frost occurs during later stages of barley development were also selected, mostly from the Middle East, Northern Africa and parts of South America. The majority of these lines were identified from passport data recorded when collection was carried out. The main information of interest included altitude, landscape and location. The majority of current mapping population parents are also included since future marker development would be greatly facilitated, if tolerance was found. Bowman isolines with distinct morphological traits particularly head structure were also selected for screening to potentially provide an insight into physical barriers to ice nucleation and penetration.

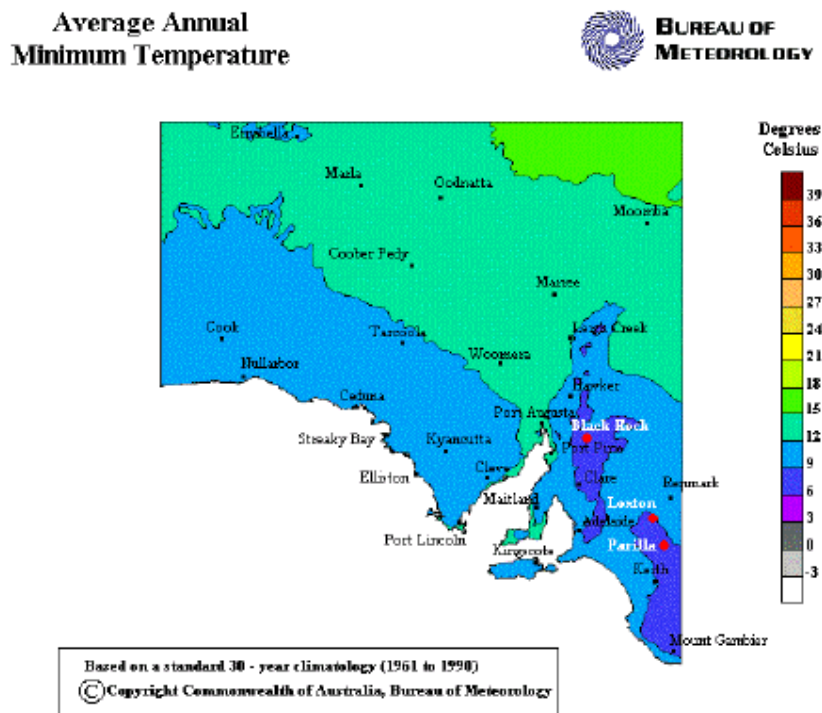
Three sites in South Australia were used for field based screening of selected germplasm. These sites were in severe (Black Rock), moderate - severe (Loxton) and moderate (Parilla) frost prone areas of the state (Figure 1). Germplasm was sown early and irrigated so flowering would occur during periods of high frost risk. This was based on long-term data obtained from the Bureau of Meteorology indicating months with the highest number of days below zero. Four seeding times over three

months were used (March - May) to maximise the chance of a discriminating frost occurring during key stages of reproductive development (Table 1).

**Table 1 Sowing dates for barley at three sites**

Seeding time	Parilla	Loxton	Black Rock
1	20/03/2001	26/03/2001	28/03/2001
2	29/03/2001	5/04/2001	6/04/2001
3	12/04/2001	18/04/2001	20/04/2001
4	26/04/2001	2/05/2001	3/05/2001

**Figure 1 Average annual minimum temperature for South Australia**



Winter barley lines had vernalisation requirements met before transplanting to the field. All winter genotypes were surfaced sterilised and pre germinated in petri dishes before placing in a cold room for four weeks (2°C). Cold treated seedlings were then potted into jiffy pots and allowed to establish for 10 days. Jiffy pots were then planted in the field at approximately the 2 leaf stage.

All entries were spaced planted 5cm apart in single rows of 1.5m. Row spacing at Loxton and Black Rock were 35cm and 50cm at Parilla. Each trial was set up as a single replicate experiment with four seeding times with Gilbert as a check every 16 rows. Gilbert was chosen as previous results indicated that it could tolerate marginally lower temperatures compared to other commercial varieties. This was concluded by one year of screening by David Woodruff at Toowoomba, Queensland. At Loxton, two blocks were sown. In one block the plants will be covered with shade cloth during the nights when air temperature falls below 2°C. Covering with shade cloth reduces the rate of radiative heat loss and effectively reduces the rate of temperature fall.

After each discriminatory frost event (between -4°C & -8°C crop temp.) emerged awns have been marked with water based paint for subsequent scoring after 10 days. Scoring of sterility, stem frosting and grain damage have been carried out on marked heads. Different colours were used for each frost so discrimination between damaging events could be achieved.

During the 1998 season a significantly late frost was observed in Victoria causing wide spread crop damage in the Wimmera region. A VIDA collaborative trial of advanced barley lines and a Tallon\*Kaputar mapping population were damaged by this frost event and damage scores recorded. Statistical analysis on damage was carried out and pedigrees compared to determine if any particular genotype was consistently observed in tolerant lines. The data were analysed within different maturity groups. A 95% confidence interval for each maturity type was calculated and outliers considered being significantly more tolerant/susceptible to frost damage compared to the majority of the population. Tallon\*Kaputar data from Victoria was combined with data collected after another frost event that affected a Tallon\*Kaputar mapping population in WA. Molecular mapping of frost damage and maturity data from these traits is currently underway.

## Results and Discussion

The frost event effecting the 1998 Victorian collaborative trial was severe with all genotypes experiencing considerable damage ranging from 30% to 100% of grains affected. Franklin was very prominent in the pedigrees of the tolerant material. From all lines scored with Franklin in the pedigree, 43% had significantly less damage, falling below the 95% confidence interval for frost damage score. From the mid to late maturity types, where the majority of Franklin pedigrees were grouped, 48% of genotypes with Franklin in their pedigree had significantly less frost damage. It was also noted that from all of the lines that had significantly lower frost damage scores than the majority of the population, Franklin featured in 33% of those pedigrees. Although true tolerance is assumed in this observation, the effect of maturity cannot be discounted with avoidance also likely to have an effect on reduction of frost damage in Franklin derived material.

These results have prompted the inclusion of two Franklin\*Arapiles mapping populations into field trials at two sites where spring radiation frost is prevalent. Tuckey, on the eastern Eyre Peninsula and Geranium in southeast are areas where late damaging frosts occur and were the ideal sites for screening of the Franklin\*Arapiles mapping populations.

During the 2000 growing season, several late frost events occurred at various locations around South Australia. Grain was collected from Borrika in the Murray Mallee where stage 3 and stage 4 trials were exposed to a significant, late, damaging frost event causing considerable grain damage. This material was malted, with major quality parameters such as hot water extract (IOB), diastatic power and viscosity recorded and compared to non damaged material. Future collections of a range of frost damaged commercial varieties will be subject to a similar study.

As well as screening barley germplasm for tolerance to frost, another area of interest is alternative developmental patterns as means of frost escape. An in depth study of physiology and development of the diverse germplasm will enable conclusions to be made on the potential for frost avoidance mechanisms in managing late frost events. If an increase in reproductive development rates can be achieved then later developing varieties can fill grain quicker in optimum conditions, reducing the yield and quality penalties that would usually be seen in standard varieties under the same conditions.

During the 2001 screening program, several frost events have occurred causing significant damage to material during key stages of reproductive development. All three sites have had noticeable damage, particularly floret sterility. As this project is only in its infancy, no conclusive results have yet been obtained but the project has the foundations in place to have a high potential for significant findings by the end of 2001. Genetic variation for spring radiation frost tolerance in barley may exist but it will take careful investigation to identify such a complex trait.

## **Future work**

Based on the 2001 results, a subset of genotypes will be selected for detailed studies on mechanisms of tolerance. This will include the study of a range of biochemical processes and different physical and developmental characteristics that may result in the increased tolerance to a frost event.

If significant tolerance can be characterised, it will be then possible to develop mapping population(s) to identify regions of the barley genome that are associated with the expression of the specific trait.

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