# Multi-environment trial analysis: Frost sterility in wheat and barley under Australian frost-prone regions

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## Abstract

Spring radiation frost is estimated to cost Australian grain growers \$360 million in direct and indirect losses every year.

Within the National Frost Initiative led by The University of Adelaide we have benchmarked barley and wheat varieties across multiple times of sowing (2-8), regions (SA, NSW and WA) and years (2010-2019) to rank frost sterility (in excess of one hundred thousand spikes per year) based on empirical observations to improve pre-season planning for frost.

Varieties with low frost sterility overall performance (OP) and low frost sterility stability (i.e. Root Mean Square Deviation - RMSD) were less frost susceptible, with performance more consistent across all environments, while varieties with low OP and high RMSD were adapted to specific environmental conditions.

# Keywords

Hordeum vulgare L., Triticum aestivum, spike fertility, factor analytic selection tools, GEI.

# Introduction

Low temperatures during the flowering period of cereals can lead to floret sterility, yield reduction, and economic losses in Australian crops. In order to breed for improved frost susceptibility, selection methods are required to identify novel sources of frost germplasm. However, the presence of genotype by environment interactions (i.e. variety responses to a change in environment) are a major constraint to select the most appropriate varieties in any given target environment (Smith and Cullis 2018; Cocks et al. 2019). An advanced method of analysis was used for multi-environment trials (MET) that includes factor analytic selection tools (FAST) to summarise overall performance and stability to specific trait across the environments. This could deliver useful information to guide growers and plant breeding programs in providing the most appropriate decision making-strategy (Smith and Cullis 2018).

The aim of this study was to develop and analyse a robust, empirical data set of wheat and barley spikes exposed to spring radiation frost affecting spike fertility (i.e. final grain set) along multiple years, and different time of sowings (TOS) in Australian frost-prone regions.

## Methods

## Growing conditions

The twenty-six (wheat) and twenty-four (barley) experiments (frost expression experiments - FEEs) were carried out in three different frost-prone regions across Australia, namely: Southern (Sth), Western (Wst) and Northern (Nth) regions. The FEEs in South Australia (SA: 10 wheat and 8 barley), New South Wales (NSW: 8 and 8) and Western Australia (WA: 8 and 8) are referred to as a multi-environment trial (MET) data-sets labelled by state and year (e.g. nsw12, sa15 and wa17, Ferrante et al. 2021). Variety names used across sites and years are available online at the decision-making platform: FV-PLUS Frost Rankings (https://www.nvtonline.com.au/frost/).

The variety connectivity across years and states is presented in Table 1, where the numbers on the offdiagonal are the numbers of varieties in common between each pair of years or states, and the numbers on the diagonals are the total number of varieties used in that year or state.

Standard practice was used to control weeds, diseases and insects.

In-crop air temperature values (°C), accumulated rainfall (mm day<sup>-1</sup>), in-crop global radiation (MJ m<sup>-2</sup>), were recorded at daily intervals from weather stations located on the paddock or were obtained from patched point data sets (SILO, Jeffrey et al. 2001). Spring radiation frost was defined when air temperature fell below 0 °C

recorded at canopy level or 2 °C from the Stevenson screen (Bureau of Meteorology - BoM) sourced from weather stations located most close to the trials (SILO; Jeffrey et al. 2001).

analysed.											
Crop	Year	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Wheat	2010	28									
	2011	28	34					Site	NSW	SA	WA
	2012	14	18	64				NSW	108		
	2013	16	20	64	70			SA	108	194	
	2014	14	18	56	58	110		WA	94	124	157
	2015	15	19	56	60	74	118				
	2016	15	19	55	59	69	86	105			
	2017	9	13	42	44	56	66	75	84		
	2018	6	10	25	27	38	50	56	63	78	
	2019	6	10	24	26	37	46	51	58	69	80
Barley	2010	-	-	-	-	-	-	-	-	-	-
	2011	-	-	-	-	-	-	-	-	-	-
	2012	-	-	48				Site	NSW	SA	WA
	2013	-	-	47	47			NSW	44		
	2014	-	-	46	45	48		SA	44	65	
	2015	-	-	46	45	48	48	WA	44	51	52
	2016	-	-	38	38	38	38	49			
	2017	-	-	25	25	25	25	33	36		
	2018	-	-	25	25	25	25	33	34	36	
	2019	-	-	25	25	25	25	33	34	36	36

Table 1. Variety connectivity showing the number of varieties in common between each pair of years and states (inset tables) on the off-diagonal for the MET data-set, while the numbers highlighted in bold on the diagonals are the total number of varieties used in that year and state. Note: in 2010 and 2011, only SA wheat was

## Measurements and statistical methods

Sowing time between each TOS (i.e. 2-8, from mid-April to early-July depending on each FEE) was delayed by approximately 100 °C days. The increased number of sowing time (2-8) was chosen to span a wide flowering window (critical period) coinciding with the higher occurrence of frost events. Within forty-eight hours following each frost event or when multiple frost events occurred within the same week (considered in this analysis as one), 30-40 main-shoot or at least primary-tiller spikes per plot were tagged and recorded at the flowering stage using different coloured tapener

tapes. Depending on the crop and experiment, plants were also tagged at the flag leaf (barley) and booting stages (wheat). Within each experiment and TOS, varieties (and their replications) that reached flowering without matching a frost event were not tagged. However, upon a subsequent frost event, only those plants not tagged, were tagged. This methodology was repeated in all TOS. Tagged spikes were left to develop until the soft dough stage (DC83-85), after which they were individually collected, bagged, labelled and frozen to assess frost sterility (FS).

Furthermore, in wheat, the percentage of FS was calculated as the ratio between aborted grains considering only the two most proximal grain positions (G1 and G2) with respect to the rachis, and the total grains at the same grain positions (G1, G2) within spikelet and multiplied by 100 (see details in Ferrante et at. 2021). For two-row barley varieties, FS was calculated as a ratio between aborted and total grains from the central fertile spikelets along the spike and then these values were transformed for analysis using a simplified Linear Mixed Model (LMM).

The recent study by Cocks et al. (2019) reported in detail the approach used for the analysis of FEE MET data-sets, fitting a factor analytic linear mixed model (FALMM). The terms included in the FALMM address the aims of the FEEs, the experimental design of the FEEs, the longitudinal nature of the FS measurements within a FEE and also accommodated the sparse sampling of plots within FEEs. Additional terms are included in the FALMM to account for extraneous variation, such as the counter and tagger. Sources of

variation, which are associated with anatomical factors such as stage of development (SOD: booting and flowering in wheat; flag leaf emergence and flowering in barley) and relevant interactions with treatment factors and longitudinal factors were also considered in the FALMM. All models were fitted using the ASReml-R package (Butler et al. 2018) in the R statistical computing environment (R Core Team 2018).

## Results

#### Frost conditions

The percentage of occurrence of frost events across experiments, falling from 2 °C to -4 °C is shown in Fig. 1. Above 91 %, 63 % and 92 % of frost events occurred between 2 °C and 0 °C in NSW, SA and WA, respectively. Occurrence fell to 5 %, 15 % and 5 %, respectively, when analysing the temperature range between 0 °C and -2 °C (Fig. 2). Frost severity was at its highest in SA (5 %) for the range between -2 °C and -4 °C.

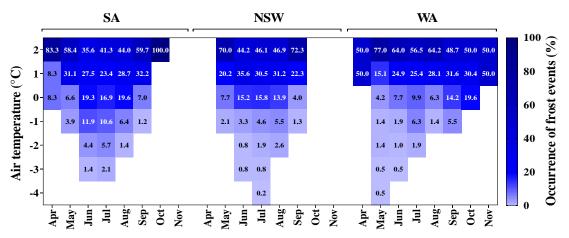


Figure 1. Frost map showing the occurrence of frost events expressed as percentage of air temperature values falling from 2 °C to -4 °C from April to November across growing seasons in SA, NSW and WA regions. The values (%) of the occurrence of frost events are shown on each categorial air temperature value.

#### Frost overall performance and frost stability

The relationship between frost overall performance (OP) and frost stability (RMSD), is the most relevant trait of the regression model which underpinned the factor analytical variance model. The OP is a measure of a variety's or breeding line's so-called average performance across all environments in the MET on the scale of the (transformed) data. In this case, varieties or breeding lines with OP values below 0 and low RMSD were less frost susceptible and performed more consistent across all environments. Varieties with a low OP and high RMSD are more likely to be adapted to only specific environmental conditions (Fig. 2).

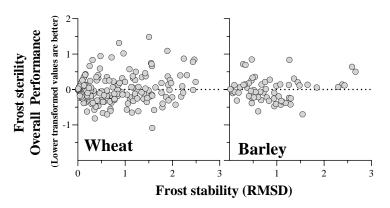
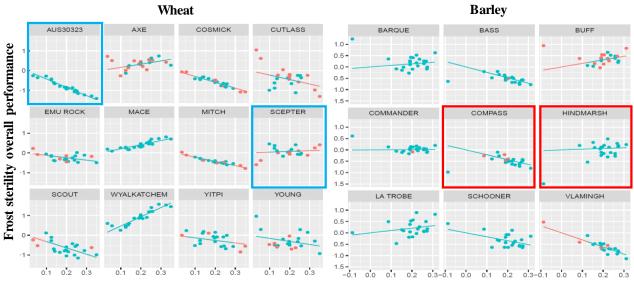


Figure 2. Empirical best linear unbiased predictors (EBLUPs) of overall performance of frost sterility (OP) as a function of root-mean-square deviation (RMSD; i.e. a measure of stability of performance across FEEs) for wheat and barley. (FAST frost rankings - https://www.nvtonline.com.au/frost/)

#### Phenotypic sensitivity to frost sterility

The OP value was the value on the regression line at the mean of the loadings (0.197 and 0.170 for wheat and barley, respectively). For example, the sensitivity of the derived synthetic line Aus30323 to a change in environment was far less than Scepter within the same maturity type (both outlined in Fig. 3). In barley, the

sensitivity of Compass to a change in environment was far less than Hindmarsh when they were compared as having a similar maturity type (to avoid phenology differences) (both outlined in Fig. 3).



Frost stability (RMSD)

Figure 3. Latent regression plots for genotypes with a range of overall performance (OP) and the root-meansquare deviation (RMSD; i.e. a measure of stability of performance across FEEs) for wheat and barley (left and right panels, respectively). Varietal sensitivity as displayed in the latent regression plots is in reference to the entire set of environments under study. Red and blue symbols correspond to FEEs where the genotype was absent or present, respectively.

## Conclusion

In this study, as far as we are aware, we showed for the first-time genotypic differences to frost damage through a MET analysis by phenotyping a vast number of accurate empirical measurements that reached in excess of 557,000 spikes. This has resulted in a substantial number of experimental units (10,317 and 5,563 in wheat and barley) across states, sites, multiple years and times of sowing.

The updated selection tools approached in this MET analysis have allowed variety comparisons with similar frost susceptibility but which have a different response to changes in the environment or vice versa. We highlight well-adapted varieties and/or lines which are allocated on the bottom left quadrant in Fig. 2 as the most promising for the industry.

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