Statistical Challenges of Analysing Frost Tolerance Trials in Wheat and Barley

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Background

- Frost damage costs the Australian grains industry over $200 million per year
- Limited progress made in breeding frost tolerant varieties
- Hard to quantify what damage is due to frost
- Large variation in timing of frosts and severity of frosts
- Critical time is when plants are flowering
- Varieties may avoid frost due to maturing earlier or later
- Statistical methods for analysis not fully developed
- Reinheimer (2004) found significant frost QTL in barley - however subsequent work to validate these QTL still inconclusive

Aim of Trials and Trait Measured

- Aim to quantify the frost tolerance of different genotypes of wheat and barley, and use this information to breed more frost tolerant genotypes.
- When frost event occurs, tillers of plants which are at flowering stage are tagged (multiple tillers per plot)
- 3 weeks later, these tillers are harvested and total number of grains and number of sterile grains are counted for each tiller
- Frost Induced Sterility (FIS) is defined as the proportion of grains which are sterile

Experimental Design

- 35 wheat varieties evaluated at Loxton 2010
- Grown at 6 Times of Sowing (TOS)- ensure that some lines are flowering at time of frost events
- 2 Replicates of each line
- Each replicate sown in a 7 row by 5 column array
- Randomised block design - replicate blocks within TOS blocks

Description of Data

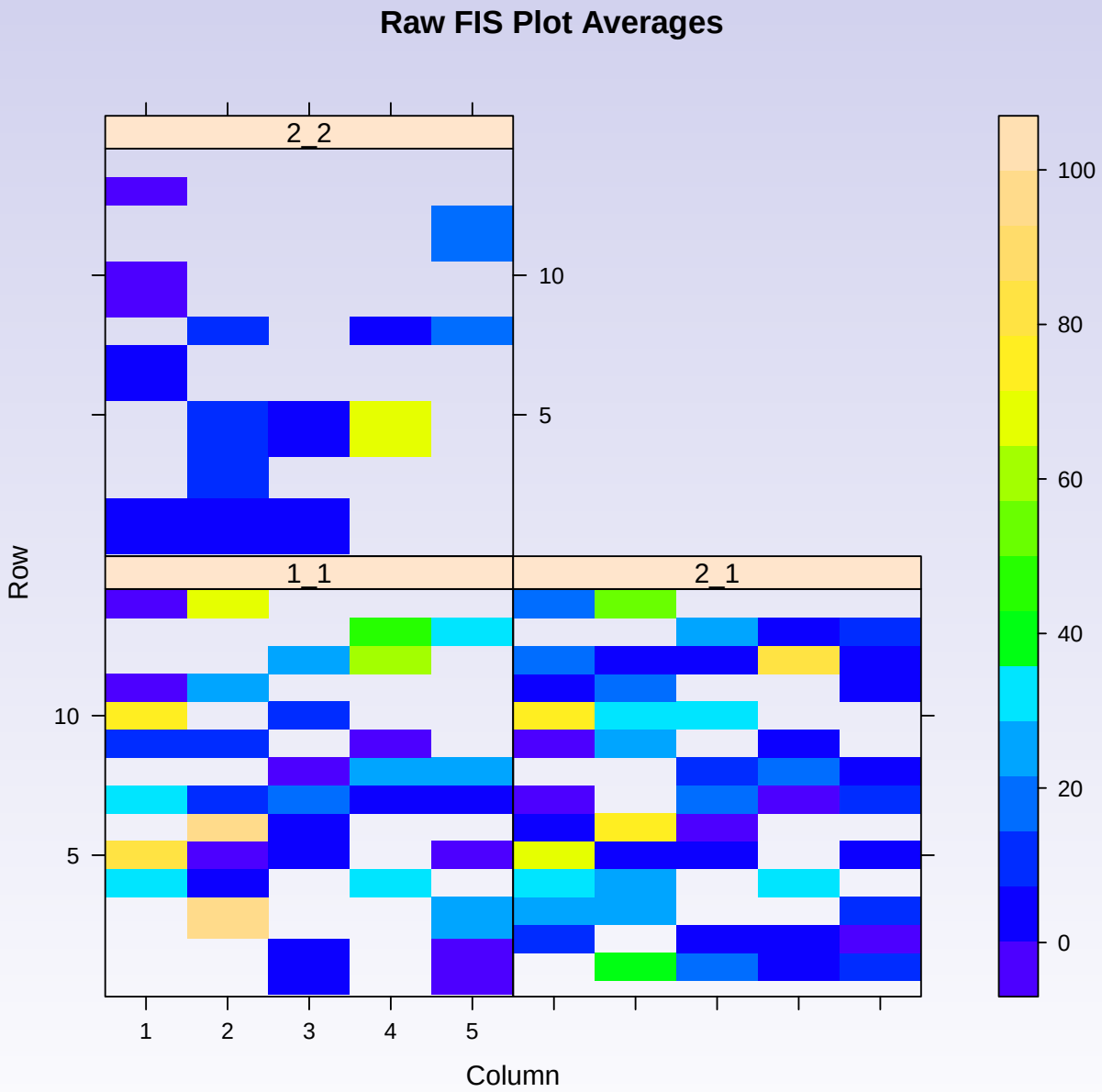
- 2 Frost Events, August 17 and August 22 2010
- Frost event 1, 34/70 plots at TOS 1 tagged
- Frost event 2, 47/70 plots at TOS 1, 14/70 plots at TOS 2 tagged
- 33 plots tagged at both frost events

Frost_TOS	1_1	2_1	2_2
1_1	24	24	10
2_1	24	27	10
2_2	10	10	11

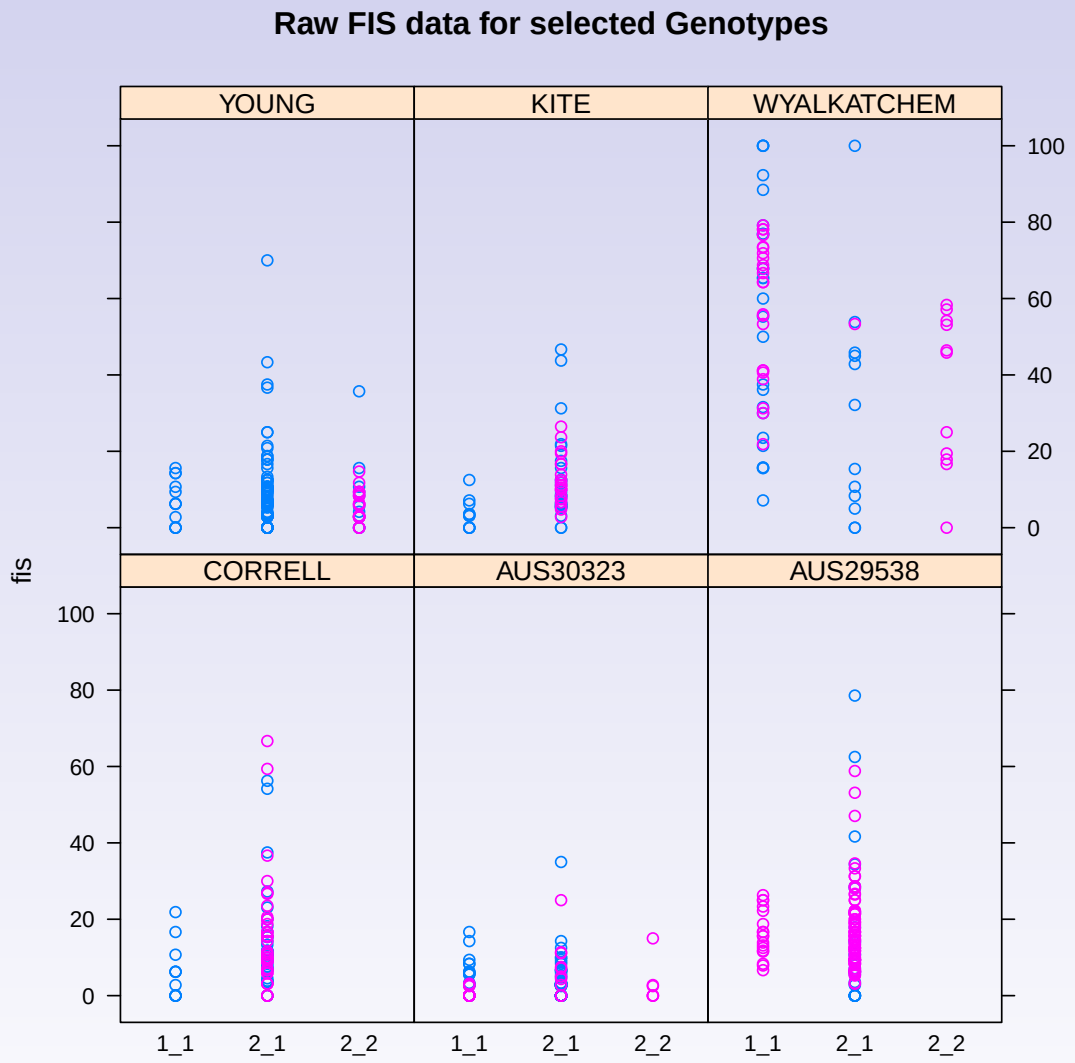
Table: Genotypes in common between Frost-TOS combinations

- Number of tillers sampled per plot ranged from 1 to 119, mean of 28.4
- Number of grains counted per tiller ranged from 14 to 46, mean of 31.5

Spatial Plot of Raw Data



Raw Data for Selected Genotypes



Transformation of Data

- Take empirical logistic transformation of data (McCullagh & Nelder, 1989) - natural transformation for binomial data
- Converts data from $[0, 1]$ to $(-\infty, \infty)$

$$lfis = \ln\left(\frac{p + 1/2n}{1 - p + 1/2n}\right) \tag{1}$$

- where p is the proportion of sterile grains, and n is the total number of grains

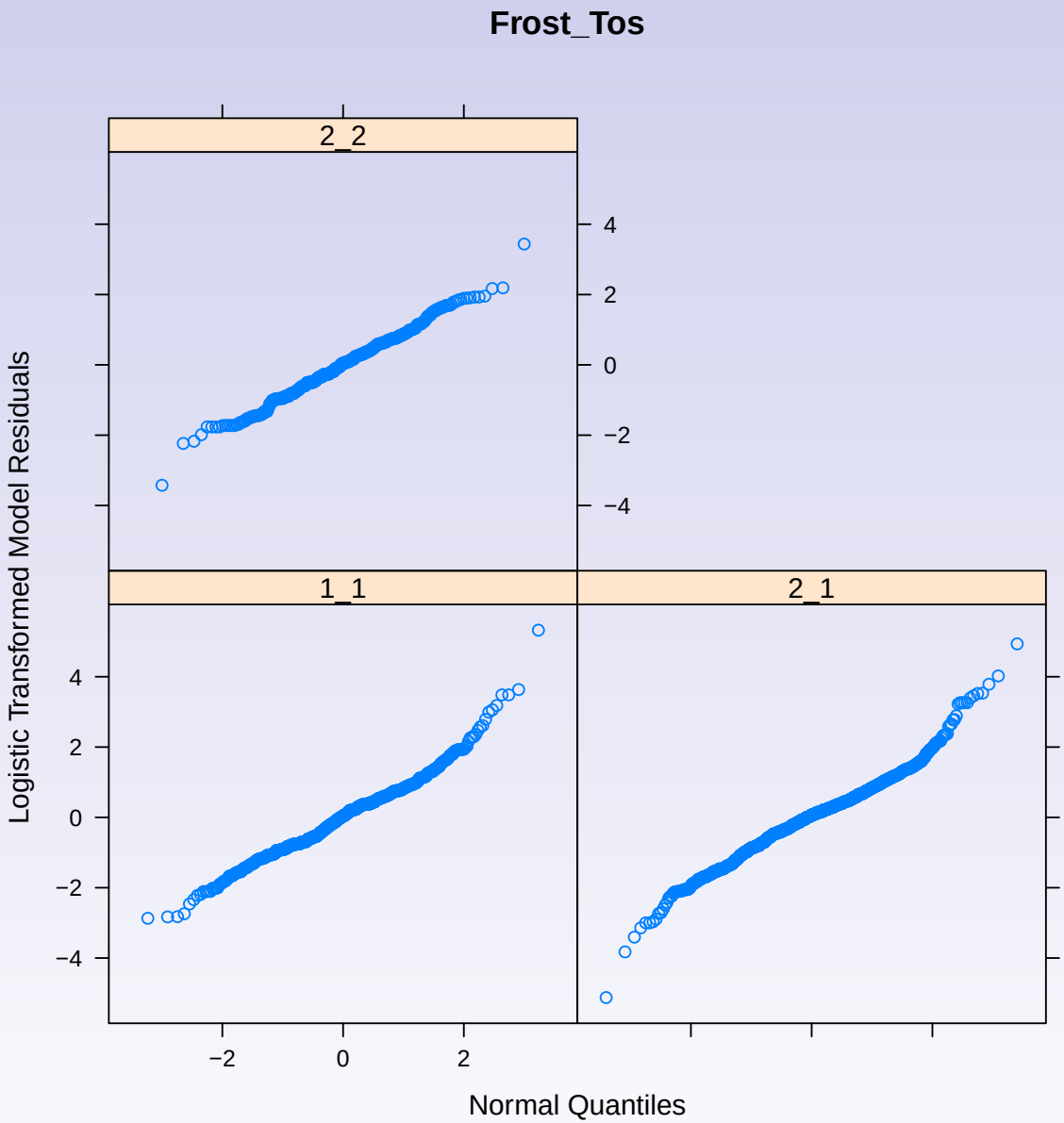
Linear Mixed Model

- Analysed using linear mixed model in ASReml-R
- Treat Each Frost by TOS combination as a different “Site” - analogous to MET analysis
- **Frost_TOS** as a fixed effect, **Genotype**, **Rep**, **Plot** as random effects
- Residual term is **Within Plot** variation
- Use factor analytic model (Smith et al. 2001) for Genotype effects if enough connected Frost_TOS with non-zero genetic variance
- Correlate plots scored at multiple frost events
- Add row and column effects if needed
- Back-transform predicted Genotype values to original FIS scale

Final Model Fitted

- Row and Column effects not significant
- Likelihood Ratio Test showed that a common correlation between sites gave the best fit

ASReml call:
loxmod <- asreml(lfis $\sim$ FrostTOS,
random= Genotype+diag(FrostTOS):Genotype
+at(FrostTOS):Rep+corh(FrostTOS):Plot,
rcov=$\sim$at(FrostTOS):units,data=lox10)


Frost_Tos
2_2
1_1
2_1
Logistic Transformed Model Residuals
Normal Quantiles
4
2
0
−2
−4
−2
0
2

Results Summary

- genetic correlation between “Sites” of 0.73
- residual correlation between Plots of -0.38
- Row and Column effects not significant

Frost_TOS	1_1	2_1	2_2
Genotype	55.2%	47.2%	48.5%
Rep	0.0%	0.4%	0.0%
Plot	10.0%	9.9%	17.5%
Within Plot	34.8%	42.5%	34.0%
Mean FIS	23.1	19.1	17.1

Table: Percentage of Variation by Frost_TOS

	1_1	$P <$Wyal	2_1	$P <$Wyal	2_2	$P <$Wyal
AUS30323	1.19	1.000	2.15	1.000	0.54	1.000
EGA GREGORY	1.75	1.000	1.92	1.000	0.31	1.000
H46	2.35	1.000	3.64	1.000	2.17	1.000
RAC875	3.20	1.000	5.10	1.000	3.38	1.000
DRYSDALE	3.90	1.000	2.63	1.000	0.75	1.000
KITE	4.33	1.000	7.52	1.000	3.84	1.000
YOUNG	4.98	1.000	5.43	1.000	3.16	1.000
KRICHAUFF	5.08	0.999	3.67	1.000	1.39	1.000
CORRELL	5.76	1.000	8.57	1.000	4.52	1.000
JANZ	6.17	1.000	3.94	1.000	1.57	1.000
HALBERD	7.31	0.997	5.50	0.999	2.55	0.999
TASMAN	9.44	1.000	11.75	0.996	6.61	0.996
EXCALIBUR	11.02	0.979	8.57	0.975	4.16	0.975
ESPADA	11.87	1.000	7.84	1.000	4.05	1.000
GLADIUS	13.28	1.000	10.47	0.998	5.76	0.998
CD87	14.04	1.000	5.22	1.000	2.37	1.000
AUS29538	15.18	1.000	10.78	0.998	5.97	0.998
CRANBROOK	15.19	0.974	12.09	0.975	6.84	0.975
MACE	16.00	0.999	12.30	0.995	4.48	0.995
SOKOLL	21.53	0.998	12.12	0.996	4.55	0.996
BERKUT	23.99	0.996	18.75	0.947	16.81	0.947
LINCOLN	24.98	0.995	35.64	0.405	24.26	0.405
KUKRI	26.84	0.979	17.83	0.946	10.77	0.946
TRIDENT	39.84	0.834	12.84	0.991	7.34	0.991
WYALKATCHEM	54.46		33.08		24.34	
VENTURA	64.35	0.201	54.71	0.024	43.47	0.024
SUNSTATE	66.32	0.163	59.31	0.011	45.96	0.011
LIVINGSTON	69.48	0.123	44.76	0.156	22.10	0.156

Table: Genotype Predicted Values Summary

Loxton Barley 2010 Results

- 70 Genotypes, 7 TOS, 7 Frosts
- Less genotypes in common between Frost_TOS
- Less genotypic variation and lower correlations between Frost_TOS

Frost_TOS	1_1	2_1	3_1	3_3	4_1	4_3	4_4	6_6	6_7	7_7
Genotype	24.2%	17.8%	0.0%	0.8%	16.4%	27.3%	11.1%	46.6%	0.0%	9.8%
Rep	0.0%	0.0%	0.0%	0.9%	0.0%	0.0%	3.2%	0.6%	11.4%	17.9%
Plot	2.3%	18.3%	32.6%	3.4%	30.1%	5.9%	14.0%	20.5%	37.6%	27.5%
Within Plot	73.5%	63.9%	67.4%	94.8%	53.4%	66.8%	71.8%	32.3%	51.0%	44.8%
Mean FIS	7.6	13.2	10.8	4.1	16.8	6.3	4.4	21.0	5.6	7.7
Number of Genotypes	13	35	27	7	26	18	7	29	30	30

Table: Percentage of Genetic Variation for Barley Experiment

Alternative method of design and analysis

Randomise TOS replicate blocks (split plot design) and treat each frost event as a “site”

- TOS not confounded with (large) blocks so can estimate TOS$\times$Genotype interactions
- more genotypes with information at each “site”

However:

- Difficult to manage smaller TOS blocks in field
- Hard to estimate interaction due to missing plots - often a different set of genotypes measured at different TOS
- Different TOS can mean quite different growing conditions
- Larger spatial distance between pairs of unreplicated genotypes
- TOS effects often not of interest, so confounding with block not such an issue

Future Work

- Measure additional variables and investigate different times of tagging - aim to reduce within plot variation
- Additional experiments in frost chamber
- Evaluate same genotypes over multiple years
- Data being generated for QTL mapping populations - need to use/develop QTL software that allow for within plot measurements

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