On Efficiency of Barley Quality Testing in Australia

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- Mixed models in the analysis of multi - phase plant breeding experiments
- Analysis of the barley malting trials
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Introduction

- Most quality characteristics in plant improvement programmes are obtained from multi-phase experiments, in which the plant varieties are first grown in the field and then further processed in the laboratory.
- The mixed model approach in combination with partial replication used at all stages of such multi-phase experiments has been shown to be efficient and beneficial for achieving genetic gain in the selection process.

Objectives

- Assessment of the relative magnitude of the sources of variation in barley malting quality trials in the example of two main traits: Hot Water Extract and Diastatic Power;
- Illustration of the importance of outlier detection and use of score tests for the Alternative Outlier Model (Cullis, Verbyla and Smith, 2006);
- Discussion of the relevance of the implementation of partially replicated designs for routine use in multi-phase experiments (Smith, Lim and Cullis, 2006).

Barley Quality Testing

- Malting process

The ungerminated barley seeds are germinated by exposure to specified conditions (given moisture and temperature) and when the grain reaches a certain point in terms of synthesis of enzymes, the germination is halted by drying (kilning) the sample. The barley seeds are converted into malt. The malt is further ground, hot water added, filtered and the wort is produced.

- Main malting traits

- measured from malt: Friability, Protein, Diastatic Power, α Amylase, β Amylase, Limit dextrinase and Kolbach Index (KI);

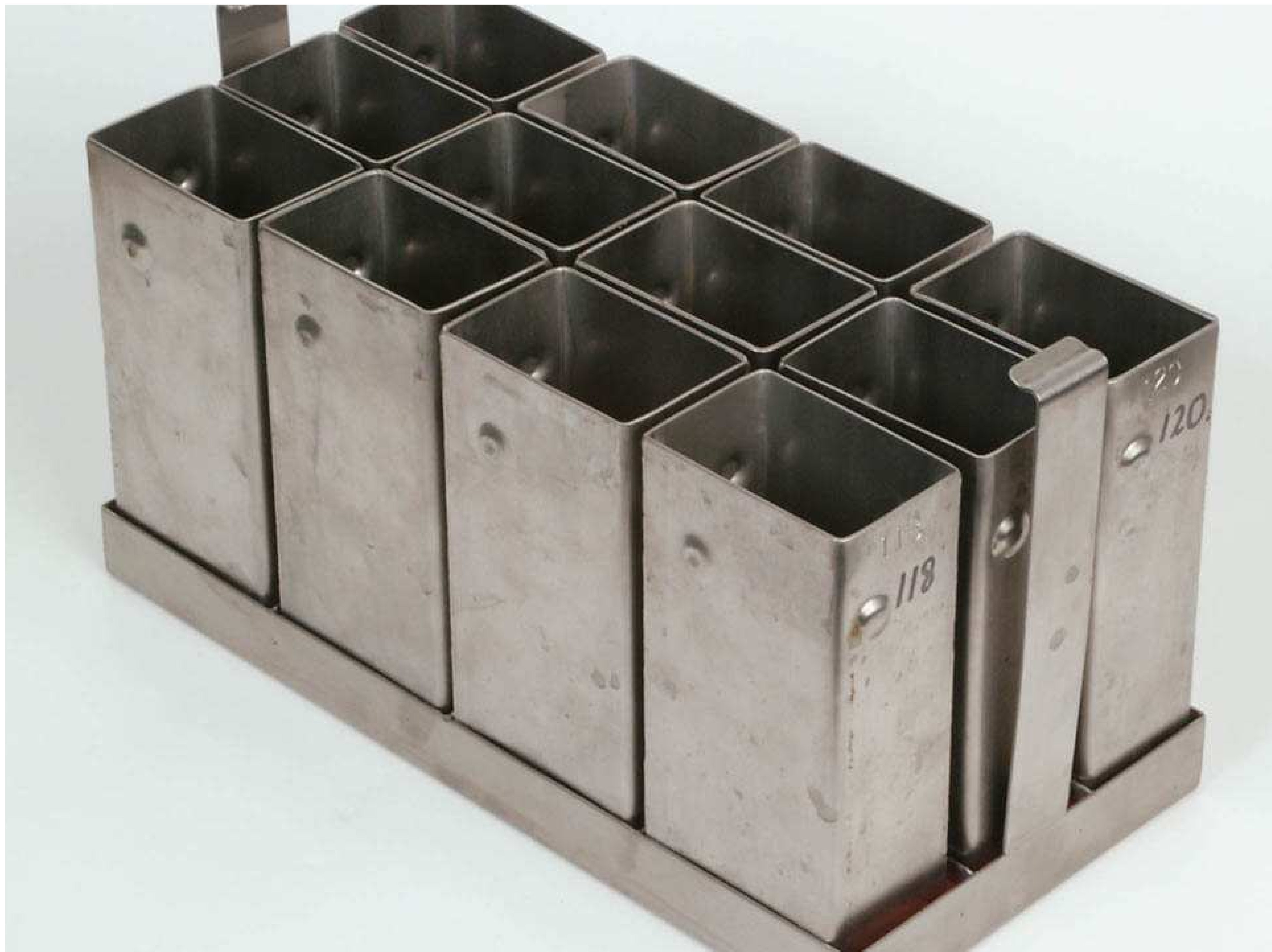
- measured from wort: Hot Water Extract, Apparent Attenuation Limit (AAL), β Glucan, Viscosity, Wort Colour and Free Amino Nitrogen (FAN).

Barley Quality Testing (cont.)

Micromalters used to process the barley have different configurations and it should be taken into account when the laboratory stage of the experiment is designed and later analysed. Three types of micromalters were used in the experiments presented here :

- a micromalter with 24 drums arranged in an 8 row by 3 column array, each drum split into two, producing 48 samples per malt run;
- a micromalter with 80 drums, arranged in a 16 row by 5 column array producing 80 samples per malt run;
- a micromalter with 96 drums, arranged in a 24 row by 4 column array, producing 96 samples per malt run.

DISPOSAL INSTRUCTIONS
NOTES
POOLE ENGLAND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Data

- Barley quality traits data from 11 trials, conducted from 2001 to 2004 by three Australian Barley Breeding Programmes were analysed and the results used to assess the variance components for key quality traits.
- Spatially balanced Row-Column designs with two or three replicate blocks were used for all field experiments. The designs were generated using DiGGer or CycDesign. The trials were laid out as rectangular arrays of plots with the number of rows varying between 16 and 39 and the number of columns between 6 and 24.
- For the laboratories, a resolvable incomplete block design was used with malting/DP days being the blocking factor. Typically, a proportion of the field plots was replicated in the laboratory. A complete replicate was process in the first half of the laboratory experiment and the other replicate in the second half.

Data summary

		Field layout				Labs layout		
		Genotypes	Columns	Rows	Reps	Drum Cols	Drum Rows	Samples [1]
PB1	Site 1	42	3	14	1	4	24	96
PB1	Site 2	42	3	14	1	4	24	96
PB2	Site 3	144	24	12	2	3	8	48
PB2	Site 4	144	8	36	2	3	8	48
PB2	Site 5	137	18	16	2	3	8	48
PB2	Site 6	137	8	36	2	3	8	48
PB3	Site 7	39	6	20	3	5	16	80
PB3	Site 8	24	6	12	3	5	16	80
PB3	Site 9	35	3	35	3	5	16	80
PB3	Site 10	39	3	39	3	5	16	80
PB3	Site 11	35	3	35	3	5	16	80

[1/] For PB1 the two sites were malted together.
For PB2 the sites were malted separately. The drums in the micromalter were split into two.
For PB3 all 5 sites were malted together.

Statistical analysis of multi-phase plant breeding experiments

- Usually the first phase in the multi-phase breeding experiment corresponds to the field trial and the second to the laboratory trial. Therefore, there are two randomizations, namely the randomization of genotypes to field plots and then the randomization of the field plots to "positions" in the laboratory process.
- A linear mixed model approach in the analysis of multi-phase plant breeding experiments proved to be efficient (Smith *et al*, 2006) and was adopted here. The model reflects the randomization processes used in each stage of the experiment and there is a residual term for each of the two phases.

Analysis of the barley malting experiments

The initial (randomization based) model used for the analysis of this set of barley malting data can be presented by the following symbolic formula:

y ~ 1 + Genotype + Frep + Fcol.Frow + Mrep

+ Mrun + Batch + Mrun.Dcol.Drow + units

where *1* represents an overall mean, *Genotype* is a factor for the genotypes, *Frep* is a factor for field replicates, *Fcol* is a factor for field columns and *Frow* is a factor for field rows, *Mrep* is a factor for the replicates in the malting process, *Mrun* is a factor for the runs of the micromalter, *Batch* is a factor for the batch in the mashing bath or for the diastase batch , *Dcol* is a factor for drum columns, *Drow* is a factor for drum rows.

Analysis of the barley malting experiments (cont.)

Term *units* is included in the model for data based on split drums in the micromalter. Term *Mrun.Dcol.Drow* becomes *units* for data without split drums.

This is a generic model and the model is modified for the three different plant breeding programmes. For the programme(s) where no information is presented about the malting replication or the mashing/diastase batches, respectively term *Mrep* or *Batch* is omitted. Similarly, *Frep* and *Fcol.Frow* are omitted in the model for the first breeding programme.

REML estimates of the variance components for DP

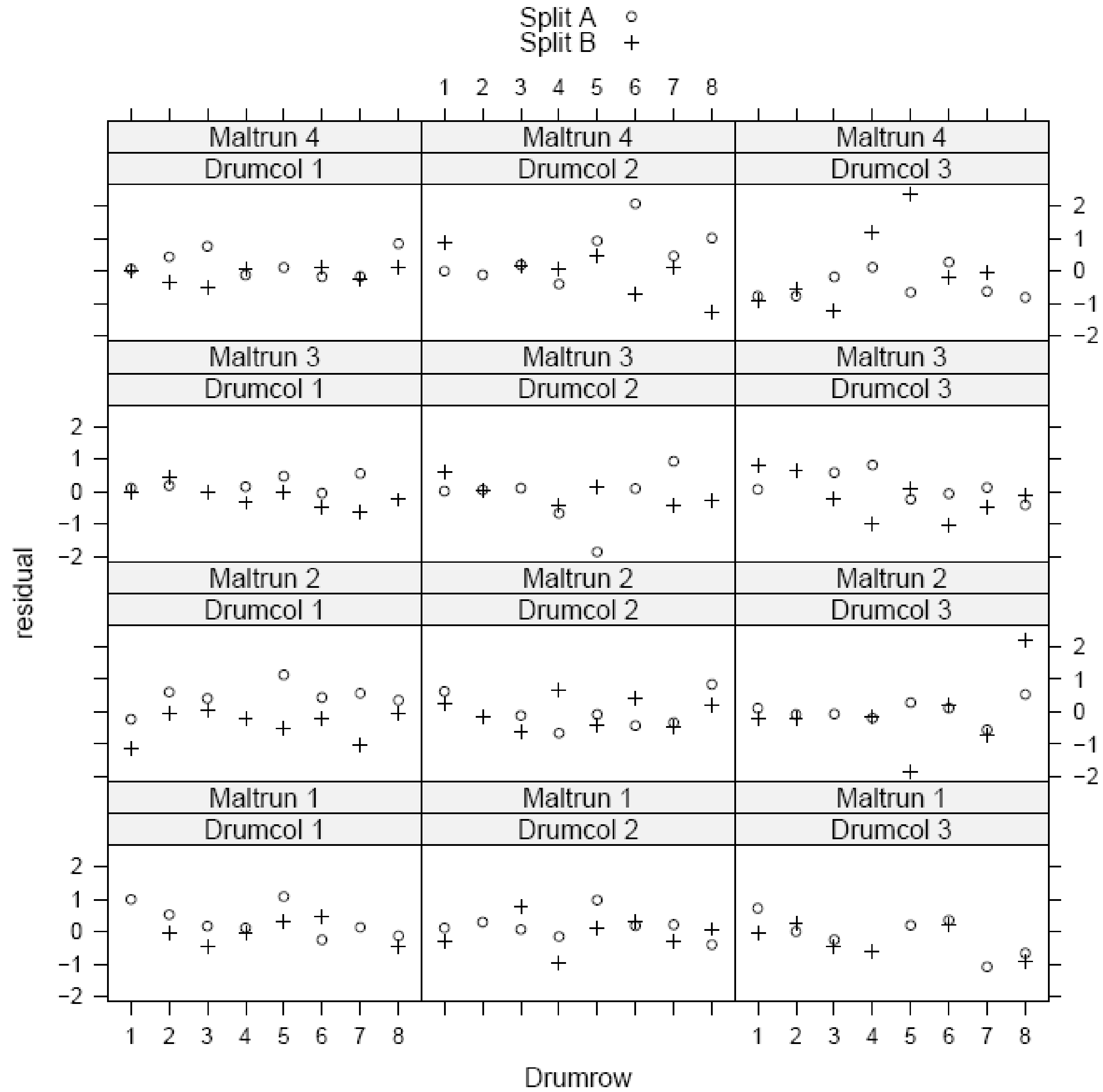
Source of Variation	PB1	PB1	PB2	PB2	PB2	PB2	PB3	PB3	PB3	PB3	PB3
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11
	2001	2001	2002	2002	2003	2003	2004	2004	2004	2004	2004
Genotype	478	2504	6834	16232	2245	3724	4438	11126	19188	4376	3880
Frep [1]	-	-	168 **(12)**	0	0	0	3239 **(152)**	0	1019 **(48)**	189 **(9)**	31 **(1.5)**
Fcol.Frow [1]	-	-	1075 **(75)**	2092 **(160)**	1004 **(245)**	511 **(269)**	1942 **(91)**	2791 **(131)**	3485 **(164)**	4535 **(213)**	0
Mrep [2]	-	-	0	0	0	0	129 **(6)**	129 **(6)**	129 **(6)**	129 **(6)**	129 **(6)**
Mrun [3]	69 **(67)**	69 **(67)**	201 **(14)**	641 **(49)**	0	339 **(178)**	141 **(7)**	141 **(7)**	141 **(7)**	141 **(7)**	141 **(7)**
Mrun.Dcol.Drow [4]	-	-	0	0	267 **(65)**	66 **(35)**	-	-	-	-	-
Batch [5]	34 **(33)**	34 **(33)**	354 **(25)**	68 **(5)**	159 **(39)**	1 **(0.5)**	-	-	-	-	-
Residual [3]	103	103	1425	1310	410	190	2130	2130	2130	2130	2130
Mean	125.8	235.4	352.5	483.3	250.1	244.2	437.2	561.9	622.9	486.4	374.3

[1/] For PB1 only one field replicate was processed.
[2/] For Sites 1, 2 there was no information about the malting replication.
[3/] Site 1-2 and 7-11 were malted (randomised) together.
[4/] *Mrun.Dcol.Drow* was fitted only in the model for PB2, where the drums were split.
[5/] For PB3 there is no *Batch* information.
The values in ( ) present the variance components expressed as a percentage of the residual variance at each site.

REML estimates of the variance components for HWE

Source of Variation	PB1	PB1	PB2	PB2	PB2	PB2	PB3	PB3	PB3	PB3	PB3
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11
	2001	2001	2002	2002	2003	2003	2004	2004	2004	2004	2004
Genotype	1.875	2.057	0.782	0.542	0.326	0.888	0.655	0.943	1.143	0.681	1.231
Frep [1]	-	-	0	0	0.291 **(51)**	0	0.474 **(409)**	0	0.104 **(90)**	0	0.095 **(82)**
Fcol.Frow [1]	-	-	0.193 **(34)**	0.386 **(143)**	0.605 **(106)**	0.178 **(51)**	1.241 **(1070**	0.687 **(592)**	0.582 **(502)**	1.077 **(928)**	0.437 **(377)**
Mrep [2]	-	-	0	0.017 **(6)**	0.006 **(1)**	0	0	0	0	0	0
Mrun [3]	0.085 **(61)**	0.085 **(61)**	0	0.02 **(7)**	0	0	0.05 **(43)**	0.05 **(43)**	0.05 **(43)**	0.05 **(43)**	0.05 **(43)**
Mrun.Dcol.Drow [4]	-	-	0.064 **(11)**	0	0	0.085 **(25)**	-	-	-	-	-
Batch [5]	0	0	0.064 **(11)**	0.169 **(63)**	0.018 **(3)**	0.147 **(42)**	-	-	-	-	-
Residual [3]	0.139	0.139	0.563	0.270	0.573	0.347	0.116	0.116	0.116	0.116	0.116
Mean	83.95	79.41	79.65	77.73	77.05	77.60	79.24	78.96	80.14	80.66	80.06

1/ For PB1 only one field replicate was processed.
2/ For Sites 1, 2 there was no information about the malting replication.
3/ Site 1-2 and 7-11 were malted (randomised) together.
4/ *Mrun.Dcol.Drow* was fitted only in the model for PB2, where the drums were split.
5/ For PB3 there is no *Batch* information.
The values in ( ) present the variance components expressed as a percentage of the residual variance at each site.



Plot of residuals for HWE, Site 3
Split A
Split B
Maltrun 4
Maltrun 3
Maltrun 2
Maltrun 1
Drumcol 1
Drumcol 2
Drumcol 3
residual
Drumrow

Conclusions

- The use of linear mixed model techniques in the analysis of multi-phase plant breeding experiments was demonstrated on highly unbalanced data and allowed modelling of substantial sources of non – genetic variation.
- The non-genetic variation for the two key traits presented here showed a pattern of the field variance components being larger than the laboratory components.
- The models presented here are the "base-line" variance components models. Further modelling will involve spatial models for trend in the field and/or the micromalter. It will lead to improved predictions of genotype effects and respectively of genetic gain.

Conclusions (cont.)

- The information obtained from the analyses will be used to determine resource allocation for barley quality traits.
- There is a marked benefit from using the partially replicated designs in all phases of the breeding experiments. Their use has allowed partitioning of the non-genetic variation into field and laboratory components.
- Detection of outliers appears to be very important, particularly in the laboratory replication. The score tests for the Alternative Outlier Model proved to be efficient in the detection of outliers.

Acknowledgments

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