ATTACHMENT 2
Analysis of adult plant phenotyping of crown rot symptoms in wheat

conducted by Alison Kelly
Agri-Science,
Department of Employment, Economic Development and Innovation,
Toowoomba QLD, 4350
61 7 4639 8831

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Chapter 1

Adult Plant Ring Test

1.1 Background

The adult ring test was designed to compare the different screening methods for crown rot in adult plants developed at sites across Australia. The study seeks to quantify the variability in results between methods using a common set of thirty entries, comprised of wheat and barley lines with varying levels of response to crown rot infection. The ultimate aim of the project is to produce a robust and reliable method for high throughput crown rot screening.

The four methods in the study each implement a differing trial structure, layout and design, together with method of infection and subsequent scoring technique. These differences are documented in the report, and results for the level of crown rot infection of thirty genotypes under each methodology are compared.

In addition, material from the Wellcamp trial was assessed by most groups giving a useful comparison of rating scales without the confounding effect of experimental method.

1.2 Nature of the data

The data measurements can be dissected into two components, both of which are essential to capture disease expression. Firstly there is the incidence of the

disease, as measured by the number of tillers infected, out of the total number observed. Secondly, there is the severity of the infection as measured by the proportion of the tiller that has some form of browning. Each method has the ability to separate these components and each method combines these measurements in different ways to derive an *index of severity*.

Statistically speaking we need to classify the nature of the measurement as being either continuous or discrete. For the crown rot data, continuous measurements are those such as length of browning of the tiller, or total length of the tiller. For the CSIRO method, the ratio of these two measures forms a continuous variable, being the proportion of the tiller with browning. If the distribution of this measurement is skewed an appropriate transformation ensures that the assumptions necessary for fitting a linear model are met.

For discrete data the response is restricted to one of a fixed set of possible values, called response categories. Most methods for assessing crown rot in adult plants assign a rating for the level of browning observed in that tiller according to an underlying scale of 0-4 (DEEDI, UOS), 1-5 (SA) or 0-3 (NSWDPI). The Table 1.1 below details the description for each category.

There is an inherent ordering in the discrete categories for crown rot, but a distinction needs to be made as to whether the categories are simply ordered, or whether the distances between the midpoints of the categories are representative of separation in the measurement scale. This differentiates between a measurement scale as being either ordinal or interval. For example, is an average of two tillers with ratings of 2 and 4 equivalent to two tillers both having a rating of 3. Likewise, is a difference between categories 1 and 2 the same as the difference between categories 3 and 4. If this is not the case, then averaging across the rating scale is not appropriate. All methods rating the extent of browning along the internodes measure on an ordinal scale.

Contrary to this ordinal scale of measurement, all methods historically employ some form of averaging across the categories to derive a severity index. UOS and SA form a simple average of the rating scales for all tillers assessed while DEEDI and NSWDPI form a weighted average across the number of tillers in each category. For DEEDI, the severity index is

$$\mathsf{index} = \frac{\mathsf{cat1} * 1.5 + \mathsf{cat2} * 3.5 + \mathsf{cat3} * 6 + \mathsf{cat4} * 9}{\mathsf{total\ tillers} * 9} \tag{1.1}$$

where $\mathsf{cat}i$ is the number of tillers in category i on the (1-4) scale.

The NSWDPI index is formed as

$$\text{index} = \frac{\text{number of tillers with browning}}{\text{total tillers}} * \frac{c}{3} * 100 \tag{1.2}$$

where c is the extent of browning on the (0-3) scale.

One further differentiation between methods is that DEEDI bulks tillers across plants, whereas other methods retain the structure of sampling tillers within plants.

The next section documents the methodology, design and statistical analysis undertaken for each assessment method.

1.3 Experimental method and Statistical analysis

1.3.1 CSIRO droplet test

The CSIRO trial followed no experimental design, but contained three replicates of 30 entries, with each replicate processed in standard order (unrandomised). A replicate was comprised of trays containing 30 punnets, and up to six punnets were grown for each entry. As there is no record of the position of lines within a tray or the position of trays inside a glasshouse, trend effects could not be explored for these data.

The measurement scale was an index formed as,

severity = length of browning/tiller height

A summary of the data is given in Figure 1.1, and shows the range of the data and degree of skewness in each of the two measurements made, and in the derived index. In addition, the histogram for plant height highlights the proportion of plants with heights lower than 20cm which may be suffering plant health issues other than that induced by crown rot.

A linear model with the following terms was fitted to the severity index.

Table 1.1: The rating scale for crown rot used in each method

Method	Ratign scale	Description
UOS	0	No lesion
	1	1st internode partially lesioned
	2	1st internode fully lesioned, 2nd internode partially or fully lesioned
	3	More than 2 internodes lesioned
	4	Dead head due to crown rot
NSWDPI	0	No browning
	0.5	Partial browning of 1st internode
	1	Complete browning of 1st internode
	2	Complete browning of 1st and 2nd internodes
	3	Complete browning of 1st, 2nd and 3rd internodes
	NB	scale is incremental by 0.5
DEEDI	0	No lesioning
	1	Partial lesioning of 1st internode
	2	Complete lesioning of 1st and partial lesioning of 2nd internode
	3	Lesioning of 1st, 2nd and 3rd internodes
	4	white head due to crown rot
SA	0	no browning
	1	1-10 % browning
	2	10-25 %
	3	25-50 %
	4	50-75 %
	5	>75 %
		browning from the crown to the first internode
		(no intermediate scores)

$$severity = genotype + replicate + replicate{:}row + replicate{:}row{:}punnet$$

Preliminary analysis indicated variance heterogeneity, so all subsequent analyses were conducted on a *sqrt* scale. Caution should be taken in interpretation of these results as the absence of experimental design produces potentially biassed genotype effects.

1.3.2 DEEDI Wellcamp trial

The DEEDI trial at Wellcamp was designed as three replicates of 30 entries, randomly laid out as a 3 column by 30 row square array. Plots were doubled in size to produce sufficient material for assessment by each of the groups involved in the rating test study. In the DEEDI methodology up to 70 tillers were assessed for each plot by counting the number of tillers in each of the disease categories (0-4).

The measurement scale was a severity index calculated as a weighted average across categories.

A summary of the raw data is given in Figure 1.2(a).

A linear model with the following terms was fitted to the severity measure.

$$severity = genotype + replicate + replicate{:}plot$$

In addition, an ordinal regression model, (McCullagh, 1980), was fitted to the data as

$$G(prob) = genotype + replicate + replicate{:}plot$$

where *prob* is the cumulative probability that the rating for any plant is in each successive category, and G is the link function in the generalised linear model framework, (see McCullagh and Nelder, 1996). For the ordinal regression model the link function is a *logit*.

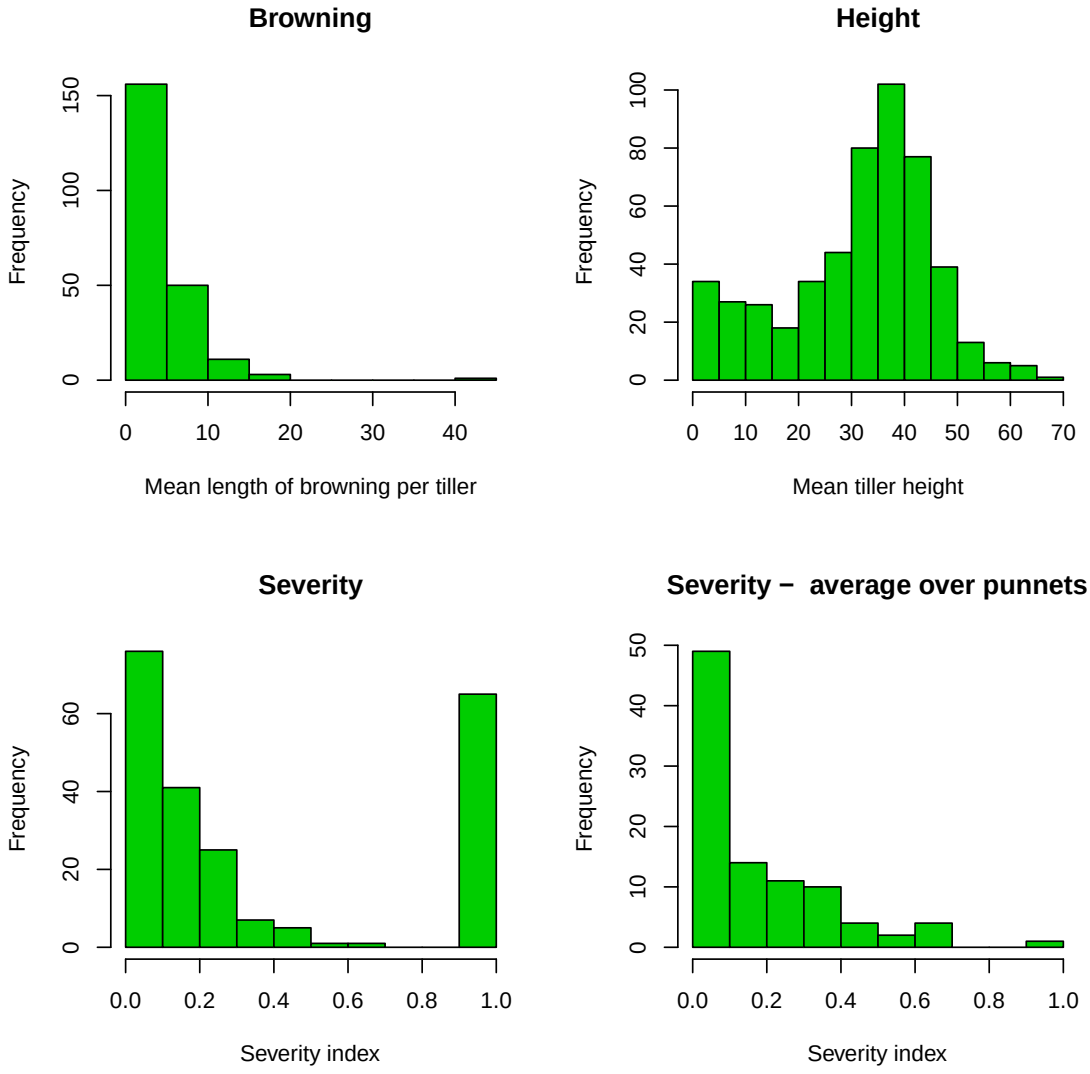

Figure 1.1: Histograms of raw data from CSIRO assessment; a) Length of browning, b) Height, c) Severity index, d) Severity index averaged over punnets

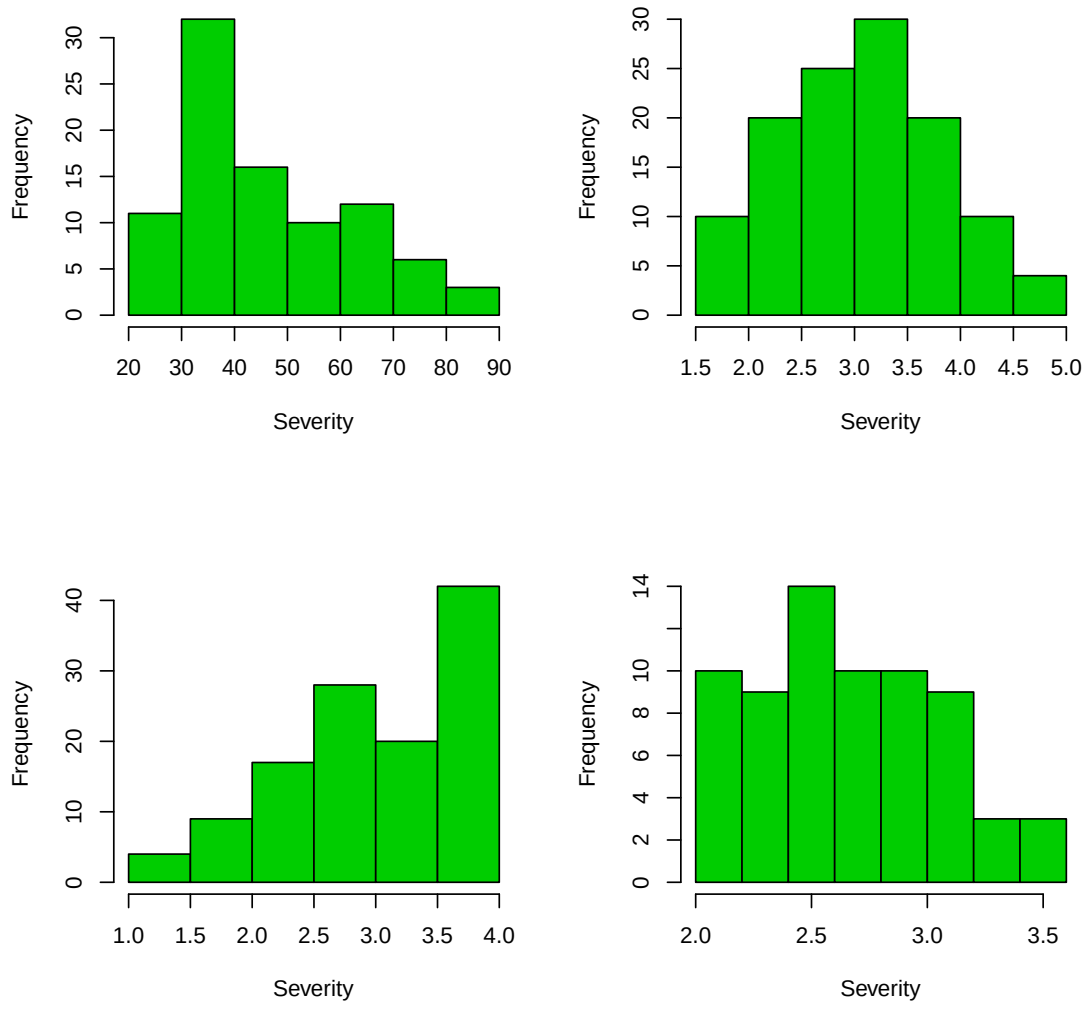

Figure 1.2: Histograms of raw data for four assessment methods; a) DEEDI pot test, b) SA terrace test, c) UOS pot test, d) UOS Field test

1.3.3 SA terrace trial

The SA trial was designed as part of the terrace trial routinely conducted in SA. It included three replicates of 30 entries, randomly allocated throughout a larger trial of 16 rows by 26 colums.

The data was a visual assessment of stem discolouration on a 1-5 scale with no intermediate categories. The score was averaged over 25 plants. A linear model with the following terms was fitted to this data.

$$severity = genotype + replicate + replicate{:}plot$$

A summary of the data is given in Figure 1.2(b).

There is some concern over the validity of these ring test results due to extreme environmental conditions experienced this season. Historic data have been provided on the terrace method and included in this report as a more accurate representation of results from this method of assessment.

1.3.4 UOS adult plant screening

Pot trial

The UOS pot trial was designed as 4 replicates of 30 entries, randomly laid out as a 6 column by 20 row array on one bench. Up to 7 plants were grown in each pot, and up to 3 tillers were assessed for each plant.

Field trial

The UOS field trial at Narrabri was designed as 3 replicates of 24 entries, randomly laid out as a 4 column by 18 row square array in the field. Up to 7 plants were assessed from each plot, and up to 5 tillers were assessed for each plant.

The data was a visual assessment of stem discolouration on a 0-4 scale where each tiller was assessed separately, and data were averaged over tillers. A linear

model with the following terms was fitted to this data.

$$severity = genotype + replicate + replicate{:}plot$$

In addition, an ordinal regression model was fitted to the data as

$$G(prob) = genotype + replicate + replicate{:}plot$$

where *prob* is the cumulative probability that the rating for any plant is in each successive category, and G is the link function in the generalised linear model framework. For the ordinal regression model the link function is a *logit*.

A summary of the raw data for both UOS trials is also given in Figure 1.2 (c) and (d).

1.3.5 Rating of Wellcamp field trial

All groups used their individual rating system to assess common material from the Wellcamp field trial. Figure 1.3 shows the distribution of the raw data for each of the scoring methods. These data enabled us to compare rating methods without the confounding influence of experimental method and environment.

The ultimate aim of this study was to compare the similarity in ranking of genotype performance between each of the methods. To achieve this, a multivariate analysis using each of the methods as traits was undertaken. Genotypes were fitted as random effects in the analysis, and estimated correlations between the methods at the genetic level were used to assess any changes in rank performance of genotypes between methods. This analysis was repeated for both method comparisons and to compare the rating systems for the common material from the Wellcamp trial.

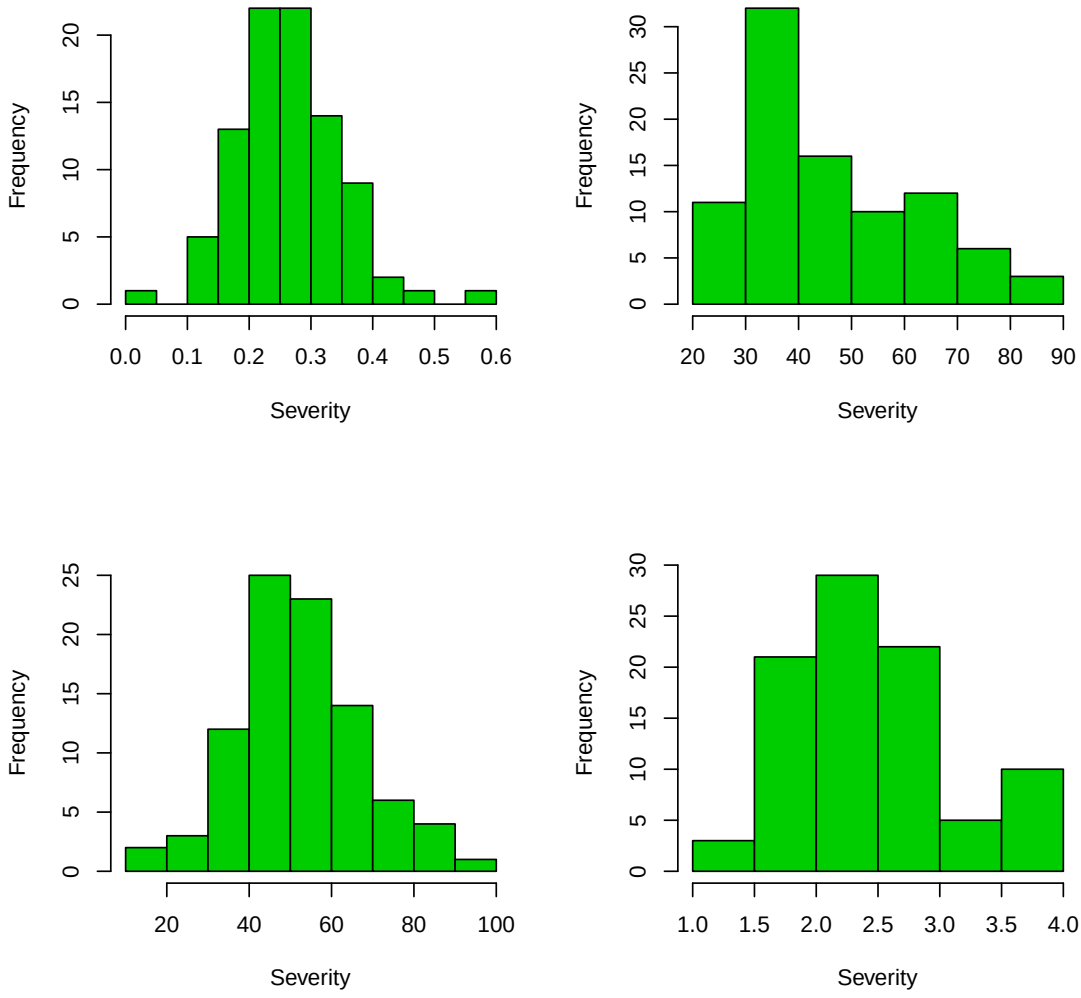

Figure 1.3: Histograms of raw data for four assessment methods of Wellcamp field trial; a) NSWDPI, b) CSIRO, c)DEEDI, d) UOS

1.4 Results

The performance of genotypes under each method is presented in the following tables, and correlations between the methods are given at the end of this section.

The results for severity index from the CSIRO droplet method are given in Table 1.2. The predicted results and standard error of difference are on the *sqrt* scale, and transformed means are also given on the data scale.

The results for crown rot severity from the DEEDI field test at Wellcamp are given in Table 1.3. The predicted results and standard error of difference are on the data scale, which is the severity index derived as a weighted average across disease categories. In addition, results from the ordinal regression on rating data are given in the Table. The regression coefficient is given for each genotype, and these can be shown to give the same genotype ranking as the analysis assuming interval data, with only very minor differences. The final column lists the probability of each genotype having a higher browning score relative to Puseas, obtained from the ordinal regression approach.

The results for crown rot severity from the SA terrace method are given in Table 1.4. The predicted results and standard error of difference are on the data scale. Furthermore, the results from the analysis of historic data have been included to provide additional information on performance of the terrace method over the past few years.

The results for crown rot severity rating from the UOS pot test and the UOS field trial at Narrabri are given in Tables 1.5 and 1.6 respectively. The predicted means and standard error of difference for severity are on the data scale. As for the DEEDI data, the rating response was also analysed using an ordinal regression approach, and regression coefficients and probability of having a higher browning score relative to Puseas are also given in these Tables. As for the DEEDI results, the changes in genotype ranks showed very little difference between the two analysis methods. As the ordinal approach supported the reliability of the genotype ranks from the analysis assuming an interval scale of measurement, comparisons between methods were made on the interval rather than the ordinal scale. It would be useful to pursue the relevance of an ordinal approach for comparing genotype response across environments for the next series of field tests.

Table 1.2: Mean crown rot severity from the CSIRO Droplet test on the ring set entries

Entry	Genotype	Severity index sqrt scale	Severity index data scale
1	Shepherd (barley)	0.213	0.045
2	Flagship (barley)	0.149	0.022
3	CPI-133814	0.113	0.013
4	Sunstate	0.254	0.064
5	Kalka (durum)	0.429	0.184
6	Wyalkatchem	0.612	0.374
7	Janz	0.581	0.338
8	Kukri	0.173	0.030
9	Gladius	0.415	0.172
10	Bellaroi (durum)	0.368	0.136
11	W21MMT70	0.374	0.140
12	CIM104	0.407	0.166
13	AUS29529	0.159	0.025
14	Puseas	0.272	0.074
15	Sunco	0.429	0.184
16	H45	0.335	0.112
17	2-49	0.458	0.210
18	Batavia	0.608	0.370
19	Yitpi	0.401	0.161
20	IRN497	0.180	0.032
21	Franklin (barley)	0.133	0.018
22	Sentinel	0.424	0.180
23	Sunvale	0.311	0.097
24	Wylie	0.127	0.016
25	E34	0.180	0.032
26	TX9425 (barley)	0.149	0.022
27	E17	0.268	0.072
28	94	0.142	0.020
29	148	0.212	0.045
30	E100-Tamaroi (durum)	0.382	0.146
LSD(5%)		0.332	

Table 1.3: Mean crown rot severity data from the DEEDI field trial on ring set entries

Entry	Genotype	Severity	Ordinal regression coefficient	Ordinal regression probability
1	Shepherd (barley)	44.53	-0.97	0.28
2	Flagship (barley)	65.80	0.30	0.58
3	CPI-133814	28.40	-3.03	0.05
4	Sunstate	40.94	-1.38	0.20
5	Kalka (durum)	68.59	0.45	0.61
6	Wyalkatchem	38.94	-1.33	0.21
7	Janz	43.94	-1.12	0.25
8	Kukri	40.76	-1.52	0.18
9	Gladius	57.96	-0.26	0.44
10	Bellaroi (durum)	68.50	0.45	0.61
11	W21MMT70	55.84	-0.63	0.35
12	CIM104	36.29	-1.63	0.16
13	AUS29529	33.78	-1.92	0.13
14	Puseas	65.77	0	0.5
15	Sunco	35.41	-1.93	0.13
16	H45	36.09	-1.70	0.15
17	2-49	21.90	-3.50	0.03
18	Batavia	47.38	-0.90	0.29
19	Yitpi	57.25	-0.21	0.45
20	IRN497	26.88	-2.85	0.05
21	Franklin (barley)	74.50	0.86	0.70
22	Sentinel	41.19	-1.56	0.17
23	Sunvale	33.79	-1.89	0.13
24	Wylie	32.91	-2.02	0.12
25	E34	43.73	-1.56	0.17
26	TX9425 (barley)	79.10	1.09	0.75
27	E17	30.79	-2.44	0.08
28	94	29.44	-2.32	0.09
29	148	39.89	-1.44	0.19
30	E100-Tamaroi (durum)	66.45	0.30	0.57
LSD(5%)		12.16		

Table 1.4: Mean crown rot severity from the SA terrace trial on ring set entries

Entry	Genotype	Severity Ring test	2008	2007	2005
1	Shepherd (barley)	3.35			
2	Flagship (barley)	3.05			
3	CPI-133814	3.30			
4	Sunstate	3.20			
5	Kalka (durum)	3.73	3.45	4.10	
6	Wyalkatchem	3.88			3.73
7	Janz	3.28	4.03	4.19	3.49
8	Kukri	2.40	2.28	3.42	2.83
9	Gladius	3.25		3.97	3.18
10	Bellaroi (durum)	2.68	4.03	4.22	
11	W21MMT70	3.10			
12	CIM104	2.63	3.74	3.84	
13	AUS29529	2.78	3.11		
14	Puseas	3.63	3.22	4.23	3.48
15	Sunco	2.93	3.29	3.50	2.94
16	H45	3.50			
17	2-49	4.54	1.72	3.01	1.55
18	Batavia	4.28			
19	Yitpi	2.98			
20	IRN497	2.38			
21	Franklin (barley)	3.43			
22	Sentinel	2.55	3.37	3.63	2.11
23	Sunvale	3.18			
24	Wylie	2.63	3.10		2.50
25	E34	3.50			
26	TX9425 (barley)	3.45			
27	E17	2.08			
28	94	2.30			
29	148	2.80			
30	E100-Tamaroi (durum)	3.43			
LSD(5%)		0.867			

Table 1.5: Mean crown rot severity from the UOS pot test on ring set entries

Entry	Genotype	Severity	Ordinal regression coefficient	Ordinal regression probability
1	Shepherd (barley)	3.98	2.23	0.90
2	Flagship (barley)	4.00	8.00	1.00
3	CPI-133814	2.60	-2.35	0.09
4	Sunstate	2.35	-3.05	0.05
5	Kalka (durum)	3.50	-0.76	0.32
6	Wyalkatchem	3.03	-1.66	0.16
7	Janz	2.48	-2.79	0.06
8	Kukri	3.35	-1.06	0.26
9	Gladius	2.73	-2.41	0.08
10	Bellaroi (durum)	2.85	-2.24	0.10
11	W21MMT70	2.95	-2.09	0.11
12	CIM104	3.83	0.75	0.68
13	AUS29529	2.45	-2.74	0.06
14	Puseas	3.78	0	0.50
15	Sunco	2.88	-2.02	0.12
16	H45	3.20	-1.31	0.21
17	2-49	3.48	-0.63	0.35
18	Batavia	3.05	-1.53	0.18
19	Yitpi	2.80	-2.27	0.09
20	IRN497	2.70	-2.43	0.08
21	Franklin (barley)	4.00	8.50	1.00
22	Sentinel	3.38	-0.90	0.29
23	Sunvale	3.68	-0.21	0.45
24	Wylie	2.85	-2.19	0.10
25	E34	3.63	-0.19	0.45
26	TX9425 (barley)	4.00	8.40	1.00
27	E17	2.65	-2.28	0.09
28	94	2.45	-2.84	0.06
29	148	2.73	-2.78	0.06
30	E100-Tamaroi (durum)	3.40	-0.88	0.29
LSD(5%)		0.85		

Table 1.6: Mean crown rot severity from the UOS field trial at Narrabri on ring set entries

Entry	Genotype	Severity	Ordinal regression coefficient	Ordinal regression probability
1	Shepherd (barley)	3.03	-0.84	0.30
2	Flagship (barley)	3.10	-0.38	0.41
3	CPI-133814	2.17	-4.66	0.01
4	Sunstate	2.53	-2.75	0.06
5	Kalka (durum)	2.33	-3.88	0.02
6	Wyalkatchem	2.60	-2.22	0.10
7	Janz	2.47	-3.04	0.05
8	Kukri	2.73	-2.07	0.11
9	Gladius	2.43	-3.25	0.04
10	Bellaroi (durum)	2.25	-3.96	0.02
11	W21MMT70	3.07	-0.60	0.35
12	CIM104	2.47	-2.88	0.05
13	AUS29529	2.30	-3.64	0.03
14	Puseas	3.23	0	0.50
15	Sunco	2.70	-2.08	0.11
16	H45	3.23	0.12	0.53
17	2-49	2.40	-3.36	0.03
18	Batavia	2.57	-2.54	0.07
19	Yitpi	3.10	-0.58	0.36
20	IRN497	2.67	-2.26	0.09
21	Franklin (barley)	3.27	0.04	0.51
22	Sentinel	3.00	-1.05	0.26
23	Sunvale	2.70	-2.12	0.11
24	Wylie	2.37	-3.46	0.03
LSD(5%)		0.51		

1.5 Comparison of Methods - Adult plant assessment

The ultimate aim of this study was to compare the similarity in ranking genotype performance between each of the methods. Table 1.7 lists the genetic correlations between each of the methods.

Table 1.7: Genetic correlation between methods for the crown rot ring set entries

DEEDI	-0.07	0.37^{wb}			
SA	-0.05	-	0.21		
UOS pot	-0.11	0.14^{wb}	0.50	0.34	
UOS field	-0.13	0.35^{wb}	0.56	0.38	0.88
	CSIRO	CSIRO^{wb}	DEEDI	SA	UOS pot

^{wb} correlations without barley

Pairwise plots between BLUPs from this analysis are given in Figure 1.4 and 1.5. The two summary plots with labelled genotype entries have been chosen to show the correlation within the pot and field forms of trialling; the first shows the relationship between genotypes BLUPs for the pot methods of CSIRO and UOS, the second shows the relationship between genotype BLUPs for the field methods of DEEDI and UOS. BLUPs for all remaining pariwise combinations of methods are shown in Figures 1.6 and 1.7.

The comparison of rating systems independent of environment is also possible as all material from the Wellcamp field trial was assessed by each group. Table 1.8 lists the genetic correlations between each of the groups rating systems when scoring the same material. Pairwise plots between BLUPs from this analysis are given in Figure 1.8.

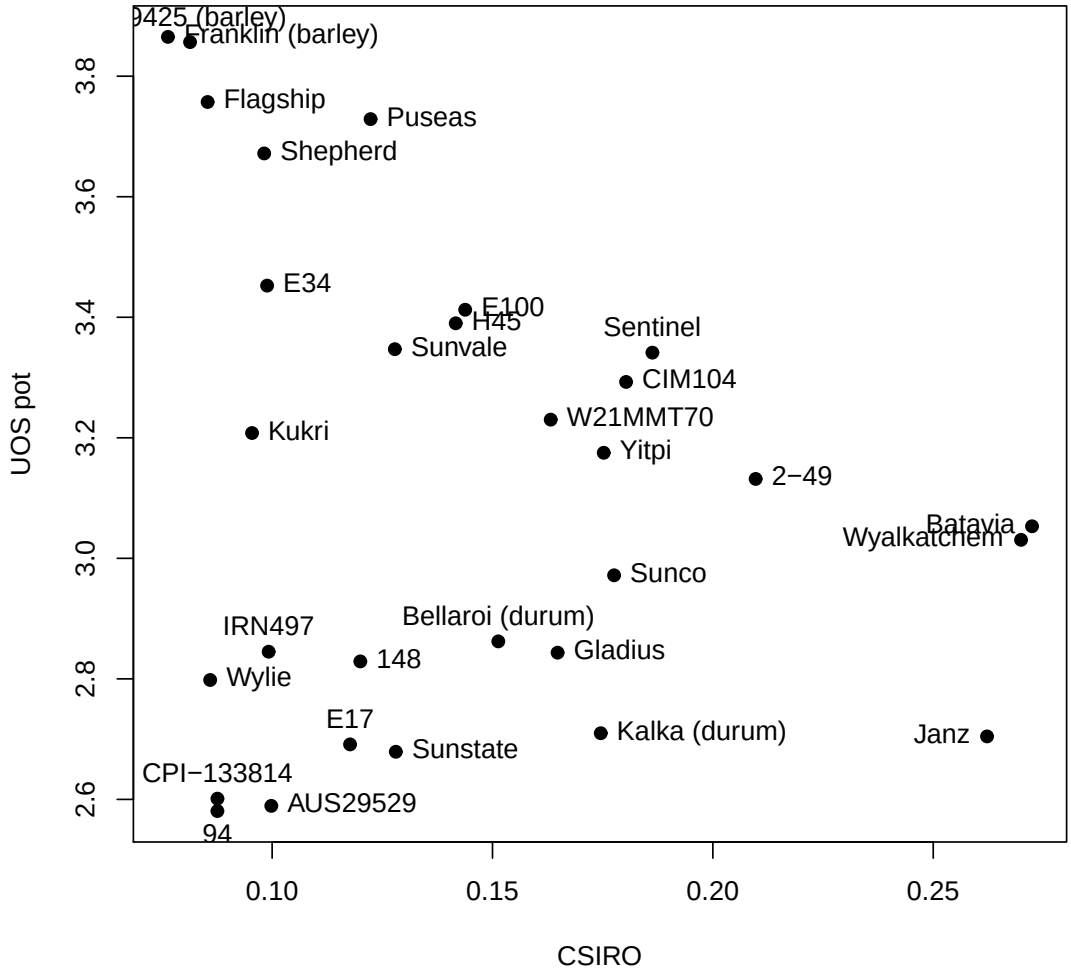

Figure 1.4: Plots of BLUPs of genetic effects for all ring set entries - UOS pot vs CSIRO pot

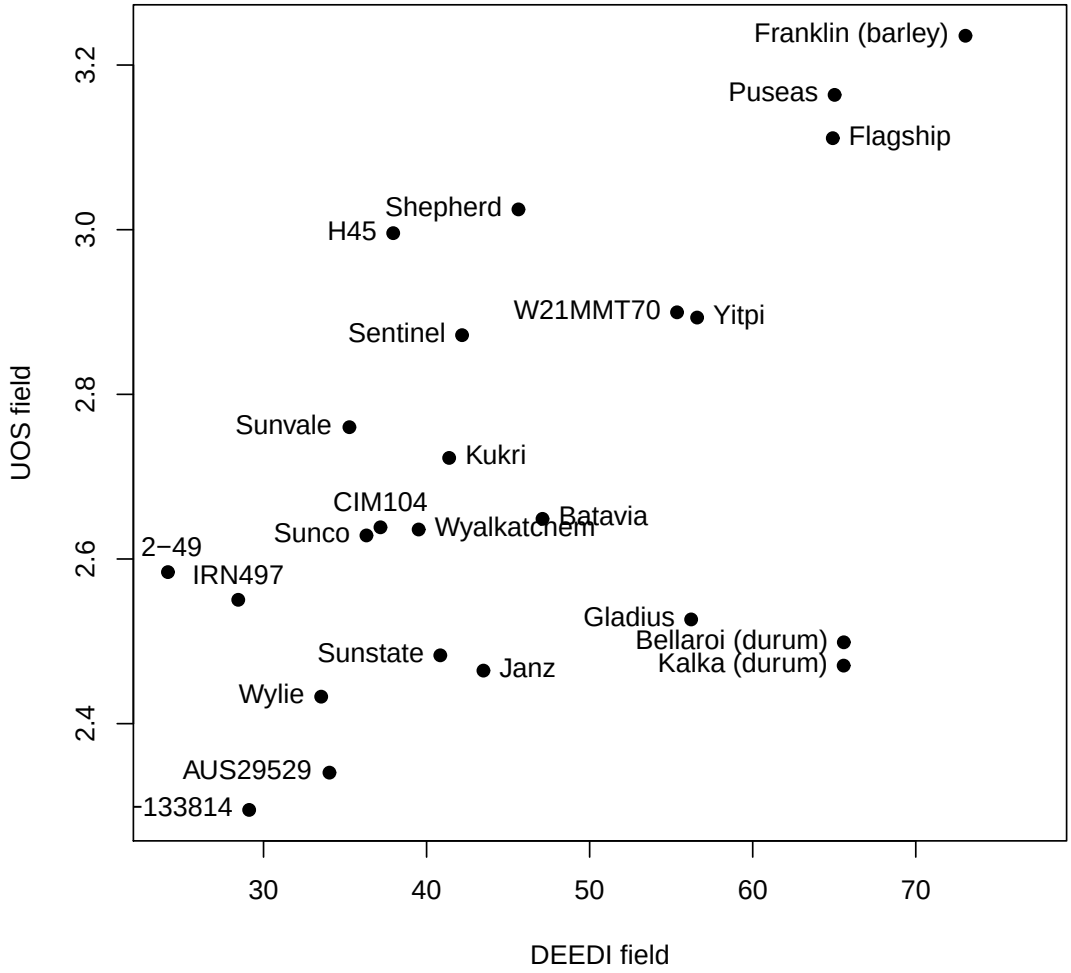

Figure 1.5: Plots of BLUPs of genetic effects for all ring set entries - UOS field vs DEEDI field

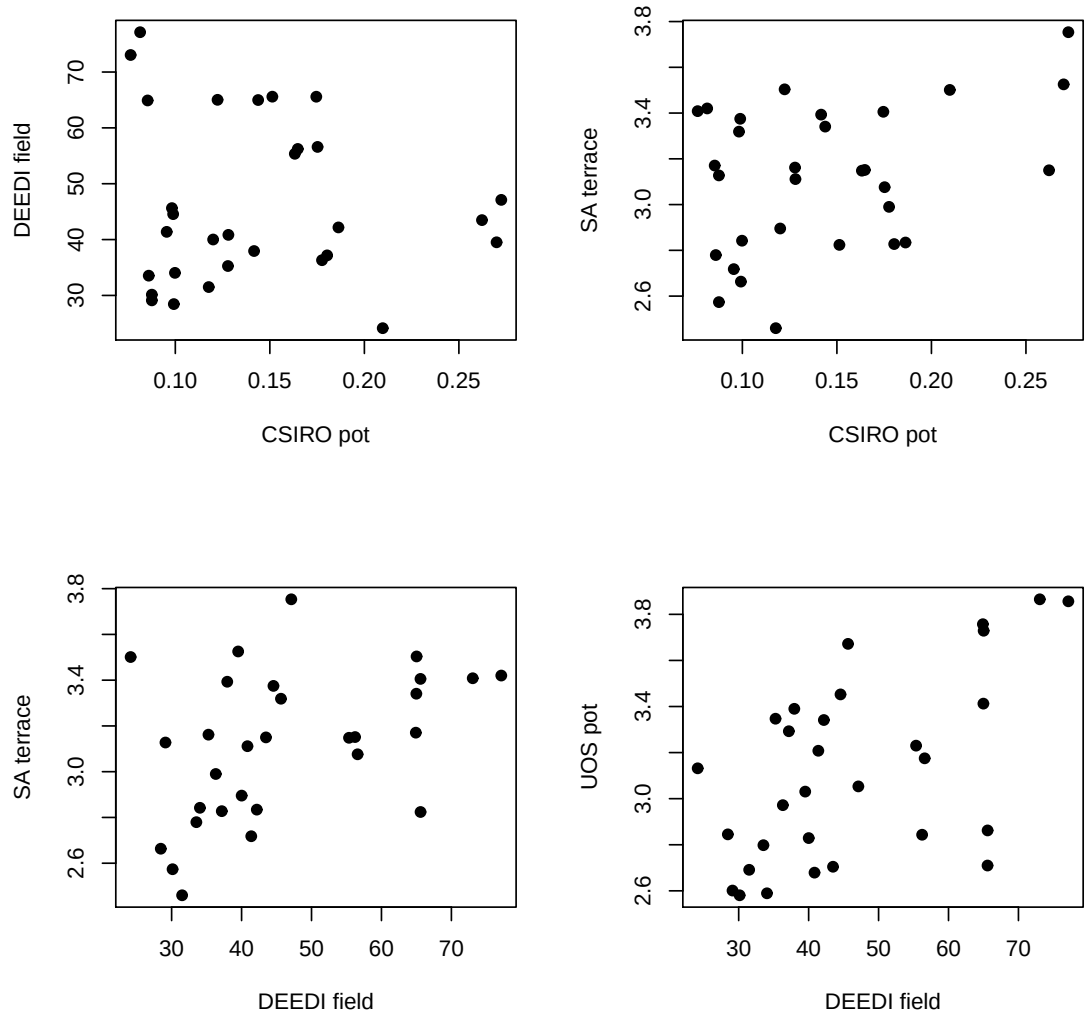

Figure 1.6: Plots of BLUPs of genetic effects for all ring set entries for a) DEEDI vs CSIRO, b) SA vs CSIRO c) SA vs DEEDI and d) UOS pot vs DEEDI

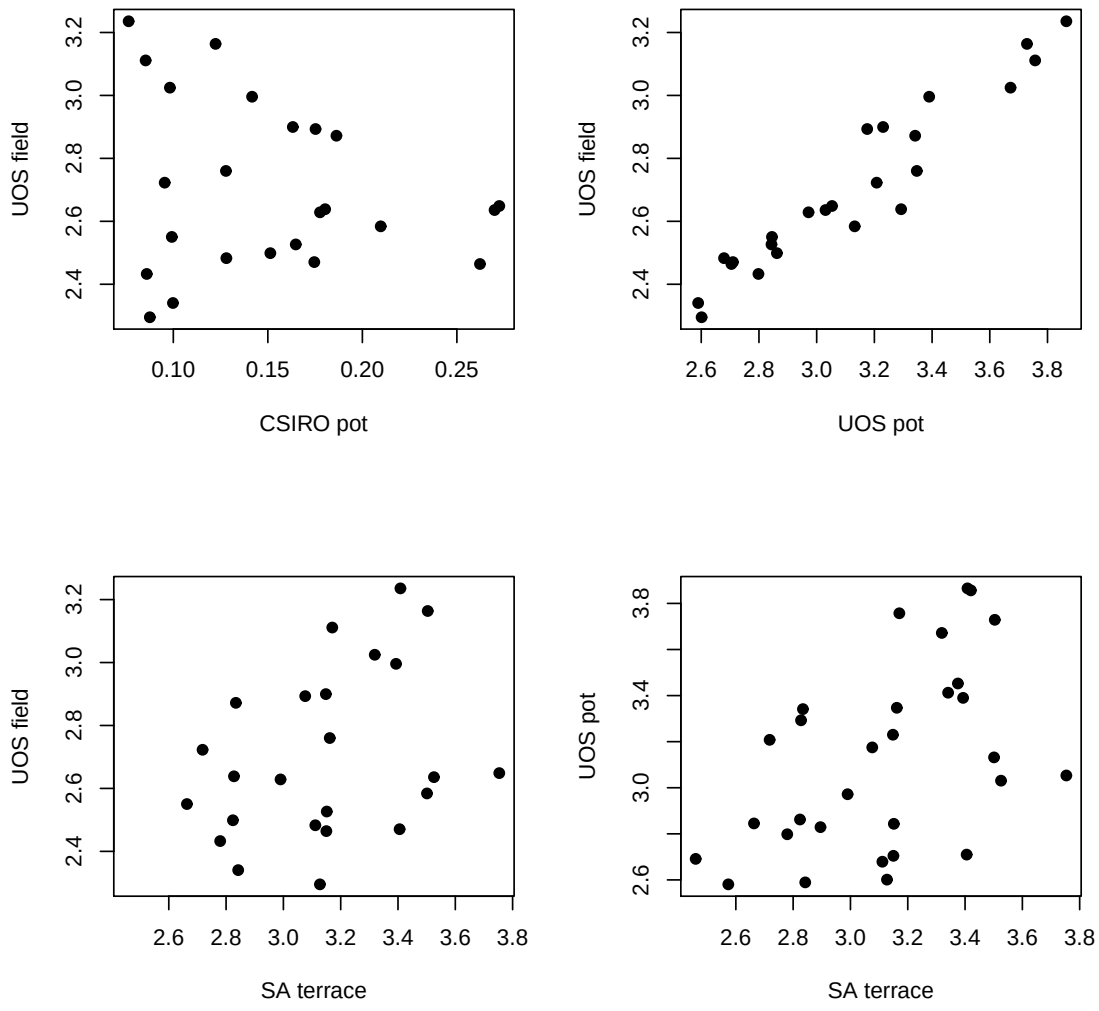

Figure 1.7: Plots of BLUPs of genetic effects for all ring set entries for a) UOS field vs CSIRO pot, b) UOS field vs UOS pot, c) UOS field vs SA terrace, and d) UOS pot vs SA terrace.

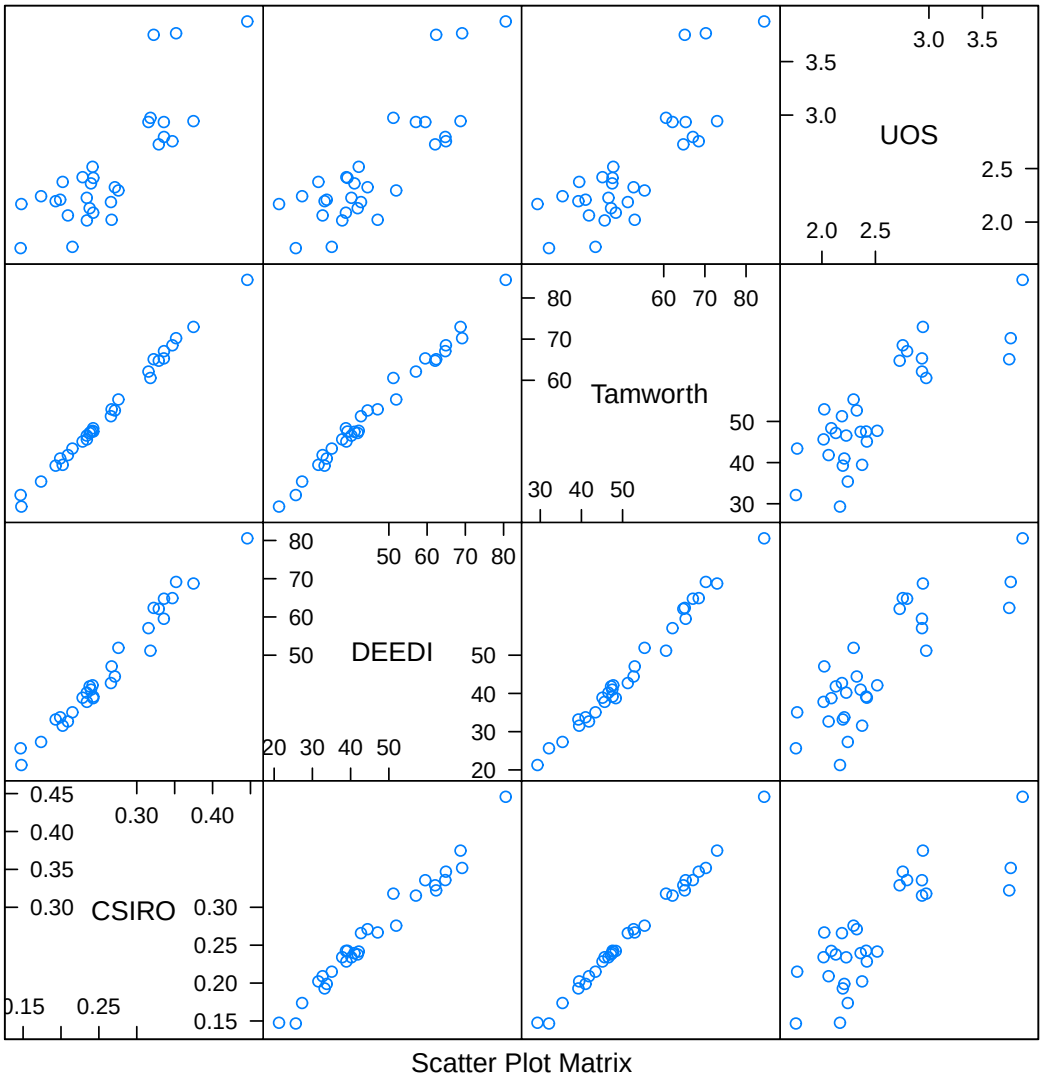

Figure 1.8: Plots of BLUPs of genetic effects between all rating methods on the Wellcamp trial

Table 1.8: Genetic correlation between rating systems for the crown rot ring set entries from the Wellcamp trial

DEEDI	0.96		
NSWDPI	0.98	0.98	
UOS	0.76	0.76	0.78
	CSIRO	DEEDI	NSWDPI

1.6 Comparison of Methods - Seedling vs Adult plant assessment

The final comparison on ring test results links the analysis of the seedling trials with the adult plant assessment. The main objective is to determine the degree of relationship between seedling and adult plant field testing. To this end the seedling tests from CSIRO, DEEDI and SA have been plotted against and correlated with the adult field tests of DEEDI and UOS, and also the adult pot test of CSIRO and UOS. Pairwise plots between BLUPs from the seedling and adult field trials at Wellcamp and Narrabri are given in Figures 1.9 and 1.10 respectively, and correlations are given in Table 1.9 for adult field trials and pot trials.

Table 1.9: Genetic correlation between seedling and adult trials for the crown rot ring set entries

	Adult field trial			
	with barley		without barley	
Seedling	DEEDI	UOS	DEEDI	UOS
CSIRO	-0.06	-0.52	0.37	-0.31
DEEDI	0.46	0.49	0.50	0.36
SA	0.33	0.08	0.41	0.19

	Adult pot trial			
	with barley		without barley	
Seedling	CSIRO	UOS	CSIRO	UOS
CSIRO	0.60	-0.34	0.51	0.08
DEEDI	-0.04	0.58	0.32	0.32
SA	-0.10	0.00	-0.12	-0.02

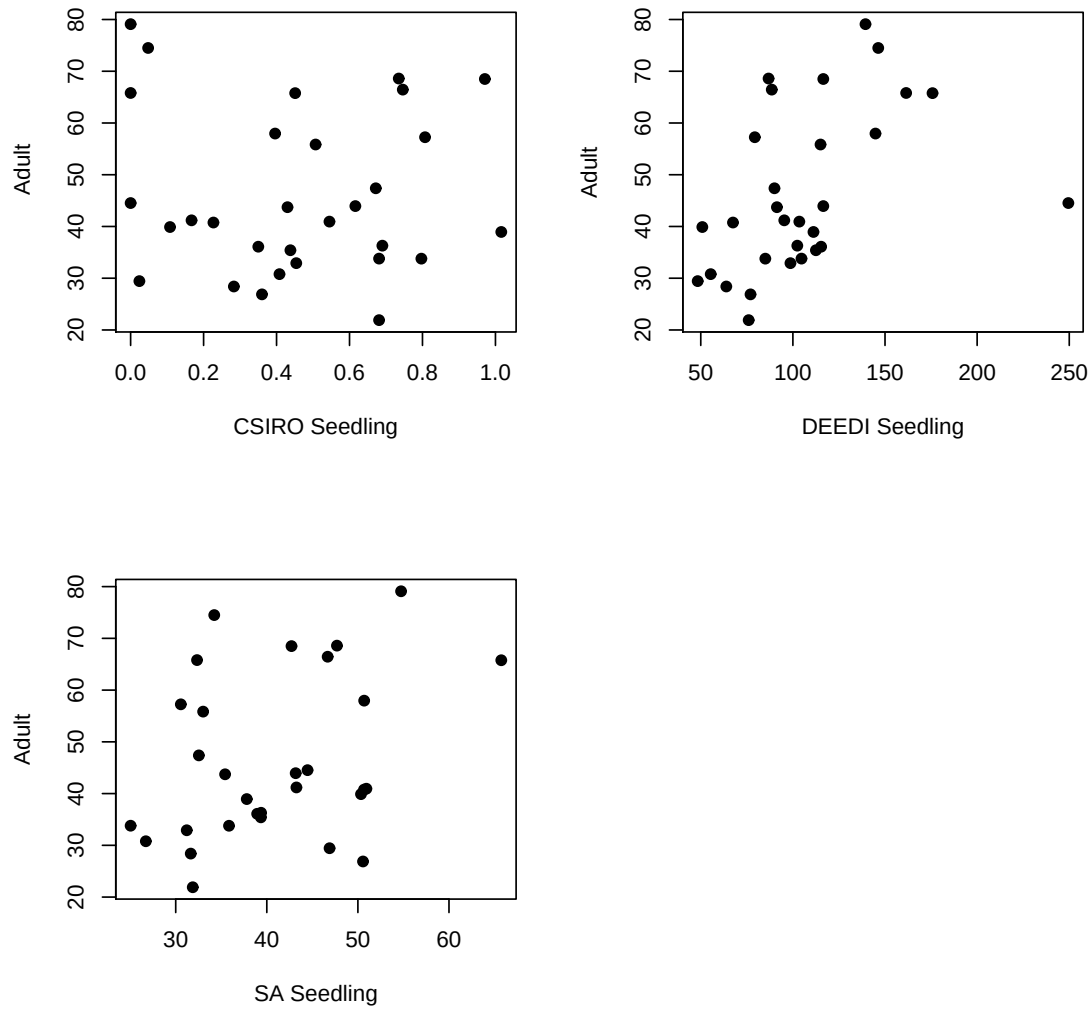

Figure 1.9: Plots of BLUPs of genetic effects between seedling trials and adult field trial at Wellcamp

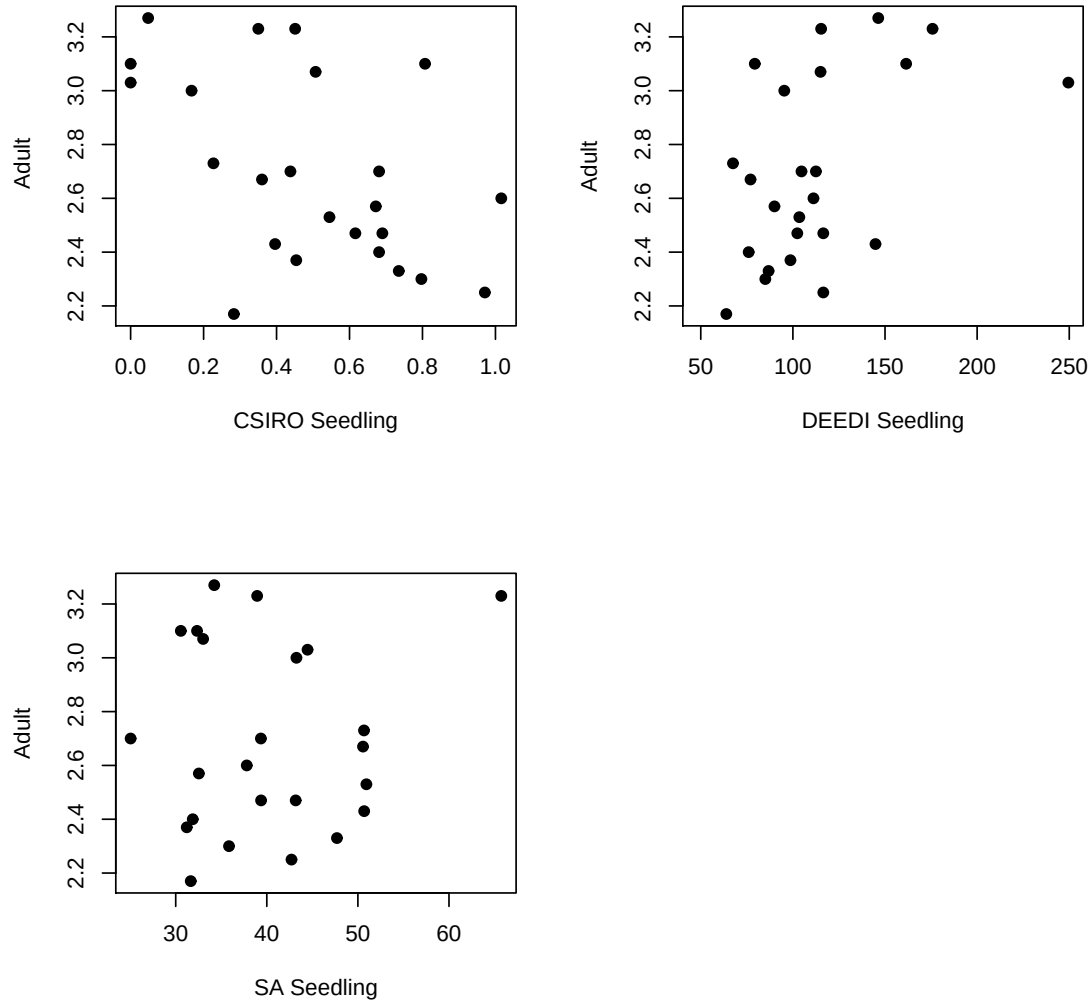

Figure 1.10: Plots of BLUPs of genetic effects between seedling trials and adult field trial at Narrabri

1.7 Discussion

- *Correlation between trials*: Correlations between DEEDI and the two UOS assessments were moderately strong, while the correlation between the UOS pot and field trial was strong. The level of correlation between CSIRO and all other methods is generally poor, and we can see that there are substantial differences in ranking of genotypes between each of the methods. This relationship was improved when barley lines were removed from the analysis.

- *SA results*: The analysis of the historic data supports the concern for the results from this years ring test. There is poor correlation between the ring test results and past years data, while there is excellent agreement in ranked genotype performance between all historic data. One qualifying factor is that the correlations are based on only up to 11 genotypes in common between any of these data sets.

- *Correlation between different methods on Wellcamp trial material*: When rating methods are compared on material from the same environment there is strong agreement in ranking of genotype performance.

- *Lack of experimental design for CSIRO method*: Efficient statistical design and analysis can achieve gains in reducing experimental error. However, no statistical analysis can account for lack of experimental design, or resurrect results from a poorly conducted/non-reproducible screening method.

- *Ordinal regression for categorical data*: Although the ordinal regression approach is more appropriate for the analysis of data from the methods using a rating scale for browning along the internodes, the resulting ranking of genotype effects varied very little from that given by an analysis assuming an interval scale of measurement, ie one based on means of category scores. This should be further explored in the next trial series, particularly in the context of analysis across multiple trials.