

# Genetic analysis of leaf rolling in wheat

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## INTRODUCTION

Leaf rolling may be associated with improved grain yield in some drought situations. Evidence of varietal differences for leaf rolling in wheat (*Triticum aestivum* L.) has been reported [1], but studies investigating the amount and nature of genotypic variation in leaf rolling of wheat are rare [2]. The aim of this work was to determine the genetic system governing leaf-rolling in wheat using two separate approaches: 1 – by analysing generation means in two bi-parental crosses; and 2 – by using a diallel-mating design.

## MATERIAL AND METHODS

### Generation-means analysis (GMA)

Lines B403D and K648R, which possess good leaf-rolling characteristics, were crossed with *cv.* Silverstar (SIL), a non-rolling line, to generate F<sub>1</sub> seeds. F<sub>1</sub> seeds were grown to derive F<sub>2</sub> as well as backcrossed to each of the original parents to generate BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub>. Six generations were available for both sets of roller×non-roller crosses: two pairs of parents, B403D and K648R (P<sub>1</sub>) and Silverstar (P<sub>2</sub>), F<sub>1</sub>, F<sub>2</sub>, and two sets of BC<sub>1</sub>F<sub>1</sub> (BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub>). Reciprocals were not considered in this study.

Individual seeds from all generations were sown at a depth of 3 cm on 12/07/05 into a 15-row by 15-column grid, spaced in both directions by 45 cm to minimise inter-plant competition, at the CSIRO Experimental Station, Canberra, Australia. The experiments were unreplicated but the entries within each experiment were replicated as follows: B403D (P<sub>1</sub>) and K648R (P<sub>1</sub>) 22 times, Silverstar (P<sub>2</sub>) 23 times, F<sub>1</sub> 20 times, BC<sub>1</sub>P<sub>1</sub> 30 times, BC<sub>1</sub>P<sub>2</sub> 30 times and F<sub>2</sub> 100 times. The two sets of line-crosses were sown in adjacent but individual experiments under rain-fed conditions. Weeds were controlled by hand and plants were sprayed to control disease.

### Diallel mating design

Fifteen wheat parents – Arrino, B403D, Carnamah, Chara, Diamondbird, Grekum 476, K648R, Kaskaskia, Kite, Krichauff, Lang, Silverstar, Tam 107, Westonia and Yitpi – were crossed in all possible combinations to produce a 15×15 diallel with reciprocals. Three main criteria dictated the choice of these parents: 1 – parents had to be representative of the Australian wheat breeding gene pool so that any interpretation of genetic effects derived from the diallel would be relevant to Australian breeding programs; 2 – parents needed to be as unrelated as possible to maximise the number of

genes independently distributed among them<sup>1</sup>; and 3 – parents needed to present variation for leaf rolling propensity.

All F<sub>1</sub> progenies and their parents were evaluated under rain-fed conditions in an experiment established on 04/07/05 at the CSIRO Experimental Station, Canberra, Australia. The experimental design was a resolvable incomplete block design, with two contiguous replicates. Each replicate consisted of 225 plots arranged in a 15-row by 15-column grid. Each plot consisted of one of the entries of the complete diallel. Each entry, represented by three seeds, was sown as hill plots. Seeds were planted by hand at a depth of 3 cm on 45 cm centres to minimise inter-plant competition. Weeds were controlled by hand and plants were sprayed to control disease.

### Traits evaluated and data analysis

At Z65, leaf-rolling scores (LRS) were recorded on two flag leaves as described by O’Toole and Cruz [3] but as modified by Sirault [2]. These flag leaves were then harvested by cutting the leaves below their leaf ligules with a razor blade. Leaves were transported to the laboratory in 15 mL Eppendorf tubes filled with water and were allowed to re-hydrate overnight under low-irradiance conditions in a constant temperature room set at 8°C. Two strips, 3 mm wide, were cut at a right angle to the mid-rib at 1/3 from the base of the flag leaf. The first strip was immersed in pure de-ionised water while the second one was immersed in a solution of polyethylene glycol 3350 (PEG). The two solutions had osmotic potentials of –0.06 and –2.0 MPa, respectively. In order to reach thermal and osmotic equilibrium, the leaf strips were incubated in the solutions for 4 h in a constant-temperature room set at 20°C. A digital image of each strip was taken using a binocular dissecting microscope and was digitised according to the method developed in Sirault [2] (Fig 1) to derive the curvature profile for each strip.

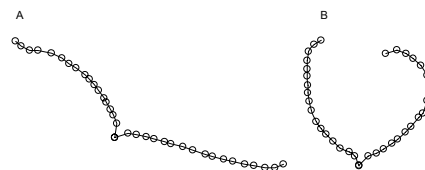


Fig 1: Digitised curves for two strips from the same leaf, (A) at –0.06 MPa, (B) at –2.0 MPa

Mean curvature<sup>2</sup> ( $\kappa$ ) was calculated and used as a trait in the diallel analysis.

<sup>1</sup> Calculation of the co-ancestry matrix indicated that the assumption of independence in gene distribution among parental lines was mostly valid.

## STATISTICAL ANALYSIS

Differences between entries ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BC_1P_1$  and  $BC_1P_2$  in the GMA, or parental lines and  $F_1$  in the diallel) were first tested using a mixed linear model fitted using Genstat V9.0 using the 'REML' directive.

Generation-means analysis was used to estimate gene effects for leaf rolling in each cross. Models were sequentially fitted starting with a simple additive model following assumptions described in Mather and Jinks [4] and were tested using the joint-scaling method.

The diallel analysis of variance was conducted according to Hayman [5] for LRS and  $\kappa$  on a plot-mean basis and was used to estimate genetic components, together with their empirical errors.

## RESULTS

### Generation-means comparisons

The mixed linear model for the two populations (B403D  $\times$  SIL and K648R  $\times$  SIL) indicated highly significant ( $P < 0.001$ ) differences between generations for LRS. LRS for B403D and K648R (4.3 and 3.8, respectively) were statistically different from Silverstar (1.8) (Table 1).

Table 1: BLUEs<sup>3</sup> value of LRS and  $\kappa$  for the GMA

	LEAF-ROLLING SCORE (score 1 to 5)	
	B403D( $P_1$ ) $\times$ SIL( $P_2$ )	K648R( $P_1$ ) $\times$ SIL( $P_2$ )
$P_2$	1.85 $\pm$ 0.05	1.83 $\pm$ 0.06
$BC_1P_2$	2.70 $\pm$ 0.08	2.82 $\pm$ 0.07
$F_1$	3.23 $\pm$ 0.06	3.63 $\pm$ 0.11
$F_2$	3.30 $\pm$ 0.05	3.23 $\pm$ 0.07
$BC_1P_1$	3.99 $\pm$ 0.07	3.49 $\pm$ 0.10
$P_1$	4.32 $\pm$ 0.05	3.80 $\pm$ 0.08
ave. sed	0.22	0.30

	MEAN-CURVATURE ( $\kappa$ ) ( $mm^{-1}$ ) for B403D $\times$ SIL	
	-0.06MPa	-2.00MPa
$P_2$	-0.067 $\pm$ 0.010	0.090 $\pm$ 0.010
$BC_1P_2$	-0.026 $\pm$ 0.008	0.171 $\pm$ 0.008
$F_1$	0.013 $\pm$ 0.008	0.223 $\pm$ 0.008
$F_2$	0.017 $\pm$ 0.006	0.245 $\pm$ 0.006
$BC_1P_1$	0.056 $\pm$ 0.009	0.341 $\pm$ 0.009
$P_1$	0.055 $\pm$ 0.010	0.376 $\pm$ 0.010
ave. sed (generation)	0.04	0.04
ave. sed (PEG)	0.05	0.05

<sup>2</sup> Curvature can be defined as the amount by which a geometric object deviates from being flat

<sup>3</sup> Best Linear Unbiased Estimator

These differences between parental means confirmed their contrasting phenotypes. In the B403D  $\times$  SIL population, means of the  $F_1$  and  $F_2$  generations were not statistically different ( $P = 0.35$ ). Meanwhile, means of  $P_1$ ,  $P_2$ ,  $BC_1P_1$ , and  $BC_1P_2$  were statistically different from each other ( $P < 0.05$ ) and from the  $F_1$  and  $F_2$  means. The  $F_1$  and  $F_2$  means were slightly higher than the mid-parent value of 2.8 in the K648R  $\times$  SIL cross, suggesting the presence of partial dominance for this cross.

The mixed linear model showed highly significant differences for  $\kappa$  in the B403D  $\times$  SIL cross, both at -0.06 MPa and -2.00 MPa. There was no interaction between  $\kappa$  and PEG treatments ( $P = 0.16$ ), indicating that the relative differences across generations were similar when increasing the osmotic stress. Silverstar presented a negative concavity at -0.06MPa ( $\kappa = -0.07 \pm 0.01$ ), whereas B403D had on average a positive  $\kappa$  ( $\kappa = 0.06 \pm 0.01$ ). At -0.06MPa,  $F_1$  and  $F_2$   $\kappa$  values were slightly higher than the mid-parent value suggesting the presence of partial dominance for the inheritance of  $\kappa$ . However, at -2.0 MPa,  $F_1$  and  $F_2$  values were very similar to the mid-parent value (0.23), suggesting that  $\kappa$  might be solely accounted for by additive gene action at higher stress.

### Model fitting

In both crosses, means for LRS showed a better fit to an additive model than an additive-dominance model (Fig2). Comparison of the  $m + [a]$  model to the  $m + [a] + [d]$  model was tested by  $\chi^2$  statistics. Neither  $\chi^2$  was statistically significant, indicating that the addition of dominance effects did not significantly improve the fit of the data.

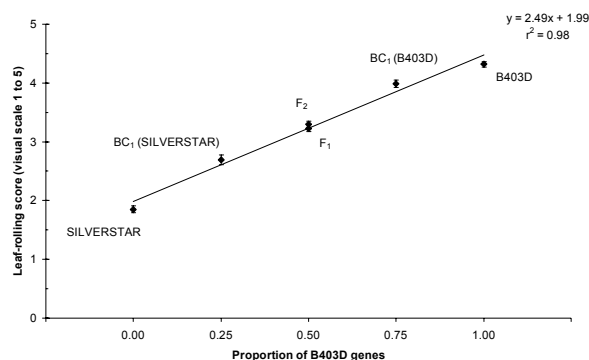


Fig 2: BLUEs ( $\pm$  standard errors) of LRS in relation to the prediction of the additive model for the cross B403D  $\times$  SIL

Coefficients of determination for the additive models were  $r^2 = 0.98$  in the B403D  $\times$  SIL cross and  $r^2 = 0.81$  in the K648R  $\times$  SIL cross, indicating clearly that an additive model was suitable to explain most of the inheritance of leaf-rolling score in these two crosses. An estimate of gene effects for the additive model was +1.2 $\pm$ 0.19 in the B403D cross, while it was +0.9 $\pm$ 0.23 in the K648R cross. The effects were calculated relative to the  $F_2$  generations.

Mean curvature was studied over the two PEG treatments simultaneously by incorporating a PEG

component in the additive model. Including extra dominant or epistatic effects did not significantly ( $P>0.05$ ) improve the fit to the model indicating that the inheritance of  $\kappa$  was additive. Gene effect was  $+0.106\pm 0.025 \text{ mm}^{-1}$  in the B403D  $\times$  SIL cross. Although PEG effects shifted the mean values of the different generations by an average of  $+0.12 \text{ mm}^{-1}$ , the additive $\times$ PEG effects were not significantly different from zero, which indicated that the additive effects were not increased or decreased with higher level of water stress.

### Diallel analysis

Hayman's analysis [5] for LRS showed significant additive 'a' and non-additive 'b' genetic variation (Table 2). The analysis of variance revealed that most of the heritable variation was accounted for by additive effects (GCA), which was almost 70% of the total sum of squares. The significance of 'b<sub>1</sub>' implied that directional dominance was present while the significance of 'b<sub>2</sub>' indicated an asymmetric distribution of genes among the parents, indicating that some alleles for leaf rolling were over-represented in certain genotypes. Significance of 'c' and 'd' suggested the presence of reciprocal effects in the diallel while the significance of 'b<sub>3</sub>' stemmed from a small number of heterotic F<sub>1</sub> hybrids.

Table 2: ANOVA according to Hayman's [5]

ITEM	D.F.	Leaf-rolling score		Mean curvature		
		M.S.	[PEG]=0.0 g.g <sup>-1</sup>		[PEG]=0.37 g.g <sup>-1</sup>	
			M.S.	M.S.	M.S.	M.S.
a (GCA)	14	22.088 ***	0.044 ***	0.084 ***		
b (SCA)	105	0.588 ***	0.002 ***	0.007 ***		
	b <sub>1</sub>	1	0.840 ***	0.002 ns	0.000 ns	
	b <sub>2</sub>	14	0.329 ***	0.002 ns	0.002 ns	
	b <sub>3</sub>	90	0.626 ***	0.002 ***	0.007 ***	
c	14	0.430 ***	0.003 *	0.010 ***		
d	91	0.529 ***	0.002 **	0.006 ***		
REPLICATE (REP)	1	2.802 ns	0.090 ***	0.006 ns		
REP <sub>x</sub> a	14	0.149 ns	0.001 ns	0.001 ns		
REP <sub>x</sub> b	105	0.087 ns	0.001 ns	0.001 ns		
	REP <sub>x</sub> b <sub>1</sub>	1	0.012 ns	0.002 ns	0.002 ns	
	REP <sub>x</sub> b <sub>2</sub>	14	0.075 ns	0.001 ns	0.001 ns	
	REP <sub>x</sub> b <sub>3</sub>	90	0.090 ns	0.001 ns	0.001 ns	
REP <sub>x</sub> c	14	0.100 ns	0.001 ns	0.001 ns		
REP <sub>x</sub> d	91	0.098 ns	0.001 ns	0.001 ns		
Total	449		0.003	0.006		

ns non-significant  
\* significant at 5% level  
\*\* significant at 1% level  
\*\*\* significant at 0.1% level

Significant additive, 'a', and non-additive, 'b', genetic variations for  $\kappa$  were present among these lines. The significance of 'b', although not large, confirmed that the differences among the 225 progenies were largely but not wholly explained by additive variation. However, additive effects were consistently the most important contributor to the heritable variance. The non-significance of item 'b<sub>1</sub>' confirmed the absence of directional dominance in both PEG treatments with  $\kappa$  in the F<sub>1</sub>. Item 'b<sub>2</sub>' was also non-significant, indicating a symmetrical distribution of genes among the 15 parental lines. However, there was evidence of non-additive deviations unique to each F<sub>1</sub> (SCA effects) as the 'b<sub>3</sub>'

item was significant ( $P<0.001$ ). This last item was the main cause of dominance effect in the diallel. Finally, items 'c' and 'd' were significant at both PEG concentrations, indicating that reciprocal effects were playing some role in the expression of  $\kappa$  in this population.

As additive genetic variance was the most important contributor to the inheritance for both LRS and  $\kappa$ , this translated into very high narrow-sense heritability:  $h^2 = 0.83$  for LRS, and  $h^2 = 0.70\pm 0.03$  at  $-0.06 \text{ MPa}$  and  $h^2 = 0.61\pm 0.03$  at  $-2.0 \text{ MPa}$  for  $\kappa$ .

### DISCUSSION

Leaf rolling ability at anthesis was evaluated using both LRS and  $\kappa$ . These traits were chosen as they proved to be the best ones for discriminating between 'good' and 'poor' leaf rollers [2]. Although measurements of the two traits were different and used different methods, similar gene action was observed. The analysis demonstrated genetic control for LRS and  $\kappa$  is essentially additive, and that dominance and allelic interaction played, at most, a minor role in the control of the characters. Heritabilities calculated in this study suggest that fixation of desirable alleles for both LRS and  $\kappa$  would be easily achieved by selection.

Given the high narrow-sense heritability of the traits, the potential for marker-based breeding may be limited. However, genome mapping could assist in chromosomal location of genes controlling the trait as well as their numbers. Good specific combining ability towards increase  $\kappa$  of a few F<sub>1</sub> in this analysis may indicate the presence of different alleles in their respective parents.

At a practical level, B403D and Kaskaskia were identified as good sources of heterotic interaction and it is from crosses involving these parents that progeny with increased leaf rolling would be expected.

### ACKNOWLEDGEMENTS

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### LITERATURE

- 1 Rebetzke GJ, Morrison AD, Richards RA, Bonnett DG, Moore C (2001). Genotypic variation for leaf rolling in wheat. Proceedings of the 10<sup>th</sup> assembly, Wheat Breeding Society of Australia, Mildura
- 2 Sirault XRR (2007). Leaf rolling in wheat. PhD thesis ANU, Canberra, Australia
- 3 O'Toole JC and Cruz RT (1979). Leaf rolling and transpiration. *Plant Sci. Lett.* **16**:111-114
- 4 Mather K and Jinks JL (1982). Biometrical genetics. 3<sup>rd</sup> edn. Chapman & Hall, London 396pp
- 5 Hayman BI (1954). The analysis of variance of diallel tables. *Biometrics* **10**:235-244