Inheritance of carbon isotope discrimination in bread wheat (*Triticum aestivum* L.)

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Summary

Reliable selection of families with increased grain yield is difficult in breeding programs targeting water-limited environments. Carbon isotope discrimination (Δ) is negatively correlated with transpiration efficiency, and low Δ is being used for indirect selection of high wheat yield in rainfed environments. Yet little is known of genetic control and opportunities for improving selection efficiency of Δ in wheat. Half-diallel and generation means mating designs were undertaken to provide estimates of the size and nature of gene action for Δ in a range of wheat genotypes varying for this trait. Significant ($P < 0.01$) differences were observed for leaf tissue Δ among parents (19.3 to 20.7‰) and F_1 progeny (19.4 to 20.9‰) in the half-diallel. General (GCA) and specific combining ability (SCA) effects were significant ($P < 0.05$), while Baker's GCA/SCA variance ratio of 0.89 was close to unity, indicating largely additive gene effects. GCA effects varied from -0.38 to $+0.34$‰ for low and high Δ genotypes 'Quarrion' and 'Gutha', respectively. GCA effects and parental means were strongly correlated ($r = 0.95$, $P < 0.01$) while directional dominance and epistasis contributed to small, non-additive gene action for Δ. Smaller Δ in F_1 progeny was associated with accumulation of recessive alleles from the low Δ parent. Narrow-sense heritability was high (0.86) on a single-plant basis. Generation means analysis was undertaken on crosses between low Δ genotype Quarrion and two higher Δ genotypes 'Genaro M81' and 'Hartog'. The F_1, F_2 and midparent means were not statistically ($P > 0.05$) different, whereas backcrossing significantly changed Δ toward the mean of the recurrent parent. Gene action was largely additive with evidence for additive $\times$ additive epistasis in one cross. Narrow-sense heritabilities were moderate in size (0.29 to 0.43) on a single-plant basis. Genetic gain for Δ in wheat should be readily achieved in selection among inbred or partially inbred families during the later stages of population development.

Introduction

Wheat crops growing in both irrigated and rainfed environments worldwide commonly experience water deficit during some stages of the crop growth cycle. The frequency and severity of soil water deficit is generally greater for rainfed wheat crops. However, changing weather patterns and worldwide water shortages will likely result in irrigated wheats being grown with less applied water, increasing the likelihood of soil water deficit (Le Houerou, 1996; Feddema, 1999). In turn, genetic improvement of grain yield under water limitation is a key objective for wheat breeders (van Ginkel

et al., 1998; Richards et al., 2002). Emphasis on selection for higher grain yield and improved performance under drought is not always successful (e.g. Cooper et al., 1997). Genetic progress is slowed owing to large genotype $\times$ environment interaction arising from seasonal differences in rainfall and drought severity. This interaction reduces heritability, thereby restricting effectiveness of empirical selection and subsequent genetic gain for yield (Feyerherm et al., 1984; Calhoun et al., 1994).

The effect of soil water deficit on crop growth varies with the timing and severity of water limitation, impacting on total biomass, grain yield and harvest

index, and the yield components grain number and size (Fischer & Wood, 1979) to reduce overall returns to growers. An understanding of physiological adaptation to water-limited environments has identified a number of drought tolerance characteristics with potential for genetic improvement of grain yield under drought (Ludlow & Muchow, 1990). However, only very few of the nominated traits have been rigorously evaluated in a breeding framework.

Genotypic increases in above-ground biomass contribute toward increased wheat yields under both well-watered and water-limited conditions (Fischer & Wood, 1979). However, yield and biomass have low heritability, and are both slow and difficult to measure, especially in early, segregating generations of a breeding program (Rebetzke et al., 2002). Transpiration efficiency (TE), the ratio of net photosynthesis to water transpired, is an important component of crop water use efficiency (biomass ÷ water used during growth) in environments where stored soil water accounts for a major portion of crop water use (Farquhar & Richards, 1984; Condon et al., 1993). Variation in TE at the leaf level is negatively related to leaf intercellular CO_2 concentration (c_i), but both TE and c_i are difficult to measure. Carbon isotope discrimination (Δ) is also associated with c_i and therefore negatively correlated with TE (Farquhar & Richards, 1984; Condon et al., 1990). Evaluation of BC_2-derived sister-lines across rainfed environments representing site-mean yields of 1 to 6t/ha, confirmed Δ is genetically correlated with aerial biomass and yield for wheat grown in water-limited environments (Rebetzke et al., 2002). Use of Δ has potential in breeding programs as it integrates TE over the period in which dry matter is assimilated and is simple to measure on large numbers of families. Studies have demonstrated that indirect selection for high biomass and yield via low Δ can be more efficient than direct selection of either trait in early generations of a breeding program targeting adaptation to water-limited environments (Rebetzke et al., 2002).

Some field studies examining the relationship between Δ and grain yield of temperate cereals have identified that this relationship can also be positive. That is, genotypic increases in Δ were associated with increases in biomass and yield (e.g. Condon et al., 1987; Voltas et al., 1999). In many cases these studies were conducted in favourable environments where water supply was not a major constraint on yield. High Δ is then associated with high leaf conductance and increased water use and growth. Thus the opportunity exists to select for high Δ where water for crop growth is plentiful. Indeed, Fischer et al. (1998) reported strong, positive relationships for Δ and yield of CIMMYT wheats representing 30 years of breeding in irrigated environments.

An understanding of inheritance of Δ is essential in development of strategies aimed at efficient selection and breeding. Repeatable genotypic variation has been reported for TE (Condon et al., 1993; Malik et al., 1999; Solomon & Labuschagne, 2004) and Δ (Ehdaie et al., 1991; Condon & Richards, 1992a; Rebetzke et al., 2002) in wheat. These reports emphasise that broad- and narrow-sense heritability of Δ is high when expressed on a plot or entry-mean basis (Condon & Richards, 1992a; Rebetzke et al., 2002). While it is understood that Δ is under strong genetic control, little is known of the nature or size of gene action for Δ in bread wheat. The objective of this study was to determine the size and nature of gene action, and heritability for Δ in wheat progeny derived from crosses between high and low Δ genotypes in half-diallel and generation means-based mating designs.

Materials and methods

Two separate studies incorporating different mating designs and a range of genotypes were undertaken to investigate genetic control of Δ in bread wheat.

Diallel analysis

Nine wheat genotypes widely-varying for Δ were selected and crossed in all possible combinations excluding reciprocals (Table 1). The wheat genotypes were diverse in origin and pedigree and contained representatives of both Australian and overseas spring wheat varieties. Parental and F_1 seed were sown singly into 30cm pots containing a standard potting mix and then placed outdoors in Canberra ACT in August 1992. Plants were watered regularly to avoid moisture stress. Average minimum and maximum temperatures during growth were 3 and 14 °C, respectively. Experimental design was a RCBD with three replications. At peak tillering (DC30 – Zadoks et al., 1974), leaf laminae were collected from all plants and dried at 70 °C for three days. Dried samples were ground to pass a 0.5mm sieve and the $^{13}C:^{12}C$ composition determined by ratio mass spectrometry using a Micromass Isochrom mass spectrometer. Carbon isotope discrimination (Δ) was

Table 1. Carbon isotope discrimination (Δ) values for parents (diagonal, **bold**), F_1 progeny (above diagonal), midparent value (below diagonal), and GCA effects from a 9×9 half-diallel mating design

Parent	Hartog	Gutha	Janz	Quarrion	Seri M82	Sundor	Sunkota	Genaro M81	Ures	F_1 cross mean	GCA effect
					‰						
Hartog	**19.9**	20.5	19.6	19.4	20.3	19.8	20.2	20.2	20.2	20.0	*−0.21***
Gutha	20.3	**20.7**	20.5	20.3	20.9	20.5	20.5	20.8	20.5	20.6	*0.34***
Janz	19.9	20.3	**19.9**	19.6	20.3	19.8	19.9	20.2	20.1	20.0	*−0.22***
Quarrion	19.6	20.0	19.6	**19.3**	20.4	19.7	20.1	20.1	20.0	19.9	*−0.38***
Seri M82	20.3	20.7	20.3	20.0	**20.6**	20.4	20.6	20.8	20.8	20.6	*0.31***
Sundor	19.9	20.3	19.9	19.6	20.2	**19.8**	19.9	20.2	20.2	20.0	*−0.20***
Sunkota	20.0	20.4	20.0	19.7	20.3	19.9	**20.0**	20.7	20.7	20.3	*0.03*
Genaro M81	20.3	20.7	20.3	20.0	20.7	20.3	20.4	**20.7**	20.5	20.5	*0.24***
Ures	20.0	20.4	20.0	19.7	20.4	20.0	20.1	20.4	**20.1**	20.3	*0.09**

*,**GCA effect is significantly different from zero at $P = 0.05$ and $P = 0.01$, respectively.

LSD (0.05) for differences among parent means is 0.36.

LSD (0.05) for differences among F_1 crosses and F_1 cross means is 0.36 and 0.22, respectively.

LSD (0.05) for $g_i - g_j$ is 0.10 where g_i and g_j are the GCA effects of the ith and jth parents, respectively.

calculated after Farquhar & Richards (1984) assuming the $^{13}C:^{12}C$ composition of CO_2 in air equals-8‰. Accuracy of the Δ measurements on the mass spectrometer was ± 0.1‰.

Analysis of variance was conducted for Δ using the SAS mixed linear models procedure Mixed (Littell et al., 1996). Genotypes (parents and F_1 progenies) were deemed fixed and replicates random effects. The half-diallel was analysed using Griffing's method II (parents, F_1 progeny, no reciprocals) (Griffing, 1956). The Diallel software of Burow and Coors (1994) was used for the diallel analysis. Both GCA and SCA effects were estimated and their relative importance determined after Baker (1978). The general linear model for the analysis was: $X_{ijk} = m + g_i + g_j + s_{ij} + b_k + \varepsilon_{ijk}$, where $X_{ijk} =$ the observed Δ value for the *ijk*th experimental unit; $m =$ the population mean; g_i and $g_j =$ the GCA effect for the *i*th and *j*th parents, respectively; $s_{ij} =$ the SCA effect of the cross made for parents *i* and *j*; $b_k =$ the effect of the *k*th block; and $\varepsilon_{ijk} =$ the residual, or error. The variance among F_1 progeny was further partitioned into additive and dominance genetic effects after Hayman (1954). Average degree and parental order of dominance was determined using the Wr-Vr graphical analysis of Hayman (1954) where Wr is the covariance between parents and their offspring in each parental array, and Vr is the variance of all progenies in each parental array. Repeatability for Δ was calculated on an entry-mean basis as the ratio of genetic to total phenotypic variance.

Generation means analysis

The low Δ wheat cultivar Quarrion (PI483063) (hereafter 'Q') was crossed in 1995 to two high Δ cultivars Genaro M81 (PI471919) ('G') and Hartog (PI483052) ('H') to generate F_1 and reciprocal cross seed. The F_1 seed was harvested and Quarrion-female F_1 seed sown in 1996 for self-pollination to produce F_2 seed, and for use as a female in backcrossing to each of the original parents to develop BC_1F_1 seed. Resulting generations for both sets of low $\times$ high Δ crosses were low Δ parent, Quarrion (P_1), high Δ parent, Genaro M81 or Hartog (P_2), F_1, F_1 reciprocal (F_1R), F_2, and backcross (BC_1P_1, BC_1P_2) generations. Individual seed of all generations were sown into 2.5×2.5cm peat pots in May 1997 and then allowed to develop two leaves in a glasshouse before transplanting into the field at the CSIRO Ginninderra Experiment Station, ACT in early July. Each peat pot was sown at a spacing of 75 cm within and between rows into an alpha-lattice experimental design containing five replicate blocks. Each block contained five plants of each parent, six F_1 and F_1R, 30 F_2 and 19 BC_1P_1 and BC_1P_2 individuals. Each cross was sown as a separate experiment. Plant nutrition and water (rainfall plus supplemental irrigation) were adequate up to, and during, the time that shoots were sampled for Δ. Single tillers were sampled in October from each plant at *ca*. DC35 (Zadoks et al., 1974), and the entire sample ground and processed for Δ as described earlier except that $^{13}C:^{12}C$ composition was

determined on a 20–20 isotope ratio mass spectrometer (PDZ Europa, Cheshire UK). Average minimum and maximum temperatures during tiller growth were 10 and 23 °C, respectively.

Analysis of variance was conducted for Δ using the SAS mixed linear models procedure Mixed, and *a priori* comparisons made between selected generation means using non-orthogonal contrasts. Generation means analysis was used to estimate gene effects for Δ in each cross. Weighted least squares regression analyses were used to solve for midparent *(m)*, pooled additive *[a]*, pooled dominance *[d]* and pooled digenic epistatic {*[aa], [ad]* and *[dd]*} genetic effects following the models and assumptions described in Mather & Jinks (1982). A simple additive-dominance genetic model containing only *m*, *a*, and *d* effects was first tested using the joint scaling test described in Rowe and Alexander (1980). Adequacy of the genetic model was assessed using a chi-square goodness-of-fit statistic derived from deviations from this model. If statistically significant at $P = 0.05$, genetic models containing digenic epistatic effects were then tested until the chi-square statistic was non-significant. Broad- (H) and narrow-sense (h^2) heritabilities and their standard errors were calculated after Ketata et al. (1976) from individual plant Δ values in each population.

Results

Diallel

The diallel experiment was deemed precise with repeatability estimated for Δ on an entry-mean basis of 0.87 ± 0.13. Large and significant ($P < 0.01$) differences were observed for Δ among entries evaluated in the half-diallel mating design (Tables 1 and 2). Parents were significantly different for Δ, ranging between 19.3‰ for the low Δ genotype Quarrion and 20.7‰ for the high Δ Gutha. The CIMMYT-developed lines, Genaro M81 and Seri M82 also had high Δ values not significantly different to the high Δ of Gutha. Genotype ranking for Δ was similar to that reported for field-grown wheat plants (Condon et al., 1993). Of all entries, Quarrion *per se* produced the smallest Δ and Gutha/Seri M82 F_1 progeny the highest (20.9‰). Mean Δ was significantly ($P < 0.01$) smaller in the parents (20.1‰) than for F_1 progeny (20.3‰) (Table 2). In most cases, F_1 Δ values were similar to calculated midparent values (Table 1). However, small yet positive midparent deviations for some F_1 progeny (e.g. Quarrion/Seri

Table 2. Analysis of variance for Δ measured on parents and F_1 progeny derived from a 9×9 half-diallel mating design in wheat. Mean squares are also provided for partitioning of crosses into general (GCA) and specific combining ability (SCA)

Source of variation	df	MS
Blocks	2	0.22**
Genotypes	44	0.48**
Parents (selfs)	8	0.68**
F_1 crosses	35	0.43**
Parents *vs* F_1 crosses	1	0.46**
Residual	88	0.04
GCA	8	2.27**
SCA	36	0.09*

*,** Indicates statistical significance at $P < 0.05$ and $P < 0.01$, respectively.

M82) suggest average heterosis and thus presence of some non-additive gene action for Δ.

Combining ability analysis revealed large and significant ($P < 0.01$) GCA and small, albeit significant ($P < 0.05$) SCA effects. Estimates of GCA and SCA variances ($\pm$se) were 0.066 ± 0.030 and 0.014 ± 0.007, respectively. Overall, GCA accounted for *ca*. 80% of the total genetic variance while the GCA/SCA variance ratio (after Baker, 1978) was 0.89, close to unity. GCA effects for Δ varied from -0.38 for low Δ genotype Quarrion to $+0.34$ for the high Δ genotype Gutha (Table 1). All GCA effects except for genotype Sunkota were significantly different ($P < 0.05$) from zero. Parent mean and GCA effects were significantly correlated ($r = 0.95$, $P < 0.01$) (Table 1). Similarly, F_1 mean and midparent values were significantly correlated ($r = 0.86$, $P < 0.01$) across the population. There were few significant ($P < 0.05$) SCA effects observed for Δ in F_1 hybrids. Positive SCA was identified for Sunkota/Ures ($+0.36$), Quarrion/Seri M82 ($+0.21$), Seri M82/Ures ($+0.21$) and Genaro M81/Sunkota ($+0.21$) whereas negative SCA effects were observed for Hartog/Quarrion (-0.27) and Hartog/Janz (-0.20). Single-plant estimates of broad- and narrow-sense heritability for Δ were 0.93 and 0.86, respectively.

Partitioning the total genetic variance following Hayman (1954) indicated large and significant additive (a) gene effects (Table 3). There was evidence of directional dominance (b_1) reflecting the earlier observation that F_1 progeny were on average higher for Δ than the mean of all parents. The significance of b_2 indicates that the extent of this directional dominance

Table 3. Mean squares for the Hayman analysis of Δ measured on parental and F_1 progeny from a 9×9 half-diallel mating design in wheat

Source of variation†	df	Mean square
a	8	2.26**
b	36	0.08**
b_1	1	0.49**
b_2	8	0.12**
b_3	27	0.06

† Source of variation is adapted from Mather and Jinks (1982) where a is the total additive genetic effect and b is the total dominance genetic effect that can be partitioned as: b_1 is the mean deviation of F_1's from the mid-parent value, b_2 is the F_1 deviation from mid-parent value over arrays, and b_3 is dominance genetic variance unique to each F_1.
**Statistically significant at $P < 0.01$.

and therefore the number of dominant alleles varies between the nine parents. There was little evidence for dominance effects specific to particular crosses (b_3), consistent with the smaller estimated SCA.

Hayman's graphical analysis was conducted to assess the genetic relationship among homozygous parents (Figure 1). In the plot of Wr against Vr most points lie in close proximity to the limiting parabola,

confirming the predominance of additive gene action for Δ. Notwithstanding this, points are close to the fitted regression line indicating the presence of some dominance gene action and reflecting the high heritability for Δ. The Wr-Vr regression coefficient was 0.73 ± 0.11 indicating the slope to be significantly ($P < 0.05$) different from zero. The estimated slope was also significantly ($P < 0.05$) different from one, indicating some inadequacy of the additive-dominance genetic model for Δ in the population, and that non-additive genetic effects could not be wholly ascribable to dominance gene action. In turn, the potential exists for epistatic gene action and/or linkage disequilibrium to be affecting Δ.

The intercept for the regression (where $Vr = 0$) was 0.06 ± 0.01 ($P < 0.01$) indicating partial dominance for Δ. The calculated average degree of dominance of 0.58 was less than 1 confirming partial dominance at each locus. The spread of points along the regression indicated differences in the frequency of dominant genes for Δ among parents. Genotypes Quarrion and Hartog contained the highest frequency of recessive alleles (fewer dominant alleles) for Δ while Gutha and Seri M82 contained the highest frequency of dominant alleles. All other genotypes were intermediate, suggesting a similar number of recessive and dominant alleles for Δ. The correlation of Wr + Vr with the common parent mean
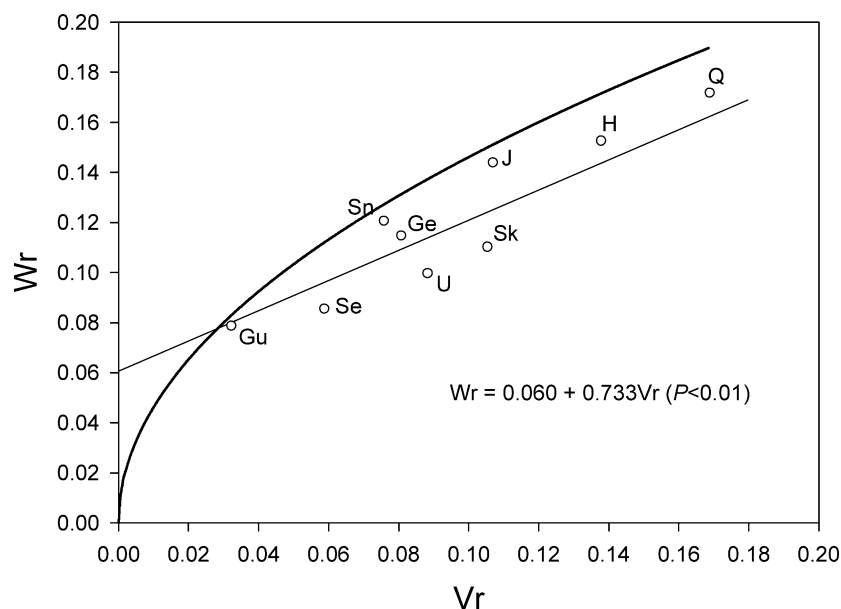

Figure 1. Covariance between parental and F_1 progeny (Wr) plotted against the variance of all F_1 hybrids in each parental array (Vr) for Δ measured in a 9×9 half-diallel mating design. Letters denote parental types: Gu = Gutha, Se = Seri M82, Sn = Sundor, Ge = Genaro M81, U = Ures, Sk = Sunkota, J = Janz, H = Hartog, and Q = Quarrion

was strong and negative ($r = -0.89$, $P < 0.01$) indicating that dominance acts to increase Δ, as suggested by the positive heterosis (b_1) described earlier.

Generation means analysis

Large and significant ($P < 0.01$) genotypic differences were observed among generation means for Δ in the two crosses of the generation means mating design (Table 4). The Δ values for the field-based, generation means analysis (17.2‰) were considerably smaller than for the half-diallel, pot study (20.2‰). These differences reflect the drier conditions at which plants were grown and Δ assessed in the field study. Nevertheless, parental differences for Δ were consistent with the Quarrion in the half-diallel producing significantly smaller Δ than either Genaro M81 or Hartog. However, the difference between Hartog and Genaro M81 was less than for the half-diallel study (*cf.* Tables 1 and 4).

Genotypic differences in Δ between parents translated into significant ($P < 0.01$) differences among progeny generations for Δ (Table 4). Both F_1 and F_2 generation means were similar to the midparent mean for the Q/G cross but deviated significantly ($P < 0.05$)

for the Q/H cross (Table 4). The F_1 and F_2 generation means were themselves similar in both crosses. Evidence for F_1 and F_2 mid-parent deviations indicated the possibility for dominance or epistatic gene action for Δ in the Q/H cross. There was some evidence for maternal effects in the Q/G cross with lower Δ associated with the Quarrion female (Table 4). Backcross generation means were significantly different ($P < 0.01$) in all crosses with the backcross mean approaching the mean of the recurrent parent. Backcross means in both crosses were statistically ($P < 0.05$) different from all recurrent parents. The phenotypic distribution of F_2 and backcross-progeny was continuous and *ca.* normal for Δ in both crosses (data not shown). Furthermore, transgressive segregation was evidenced with progeny producing significantly ($P < 0.05$) higher or lower Δ than either parent (data not shown). Distributions also indicated overlapping of backcross progeny suggesting the possibility of epistatic interactions between loci.

Generation means analysis indicated different modes of inheritance for Δ in the two crosses (Table 5). Tests for goodness-of-fit indicated a reasonable adequacy of the three parameter genetic model (m, a, d) ($P = 0.08$ and 0.06 for Q/G and Q/H crosses, respectively). Further testing revealed a simple mean and additive genetic effects model was most suitable for the Q/G cross ($P = 0.15$), whereas a more complex mean, additive and digenic additive × additive epistatic effects model was most appropriate for the Q/H cross ($P = 0.14$) (Table 5). Genetic effects in all models were significantly different from zero. In both crosses,

Table 4. Mean Δ for parental, F_1 and segregating generations in two low × high Δ wheat crosses

Generation	Cross	
	Quarrion/Genaro 81	Quarrion/Hartog
	‰	
Generation means		
Low Δ parent (P_1)	16.3 (Quarrion)	16.3 (Quarrion)
High Δ parent (P_2)	17.6 (Genaro M81)	17.4 (Hartog)
$P_1 \times P_2$ (F_1)	16.9	17.1
$P_2 \times P_1$ (F_1R)	17.0	17.1
$P_1 \times P_2$ (F_2)	17.1	17.1
$F_1 \times P_1$ (BC_1P_1)	16.5	16.6
$F_1 \times P_2$ (BC_1P_2)	17.1	17.2
LSD†	0.3	0.2
Contrasts‡		
P_1 *vs* P_2	−1.4**	−1.1**
F_1 *vs* F_1R	−0.3*	−0.1
F_1 *vs* F_2	0.17	0.1
BC_1P_1 *vs* BC_1P_2	0.6**	0.6**

† LSD (0.05) for testing differences between generation means.
‡ Estimates of effects for *a priori* single df contrasts.
* $P < 0.05$; ** $P < 0.01$ for differences between generations.

Table 5. Estimates of gene effects (± standard errors) for Δ measured on parental, F_1 and segregating generations for two low × high Δ wheat crosses

Gene effect†	Cross	
	Quarrion/Genaro 81	Quarrion/Hartog
	‰	
m	16.9 ±0.1**	17.1 ±0.1**
[a]	−0.6 ±0.3*	−0.6 ±0.1**
[aa]	−	−0.3 ±0.1*
χ^2‡	6.7	5.6
P-value	0.15	0.14

†m, estimated mean; *[a]*, pooled additive; and *[aa]*, pooled additive × additive genetic effects.
‡ Chi-square testing H_o: adequacy of additive or additive × additive genetic models.
*,** indicates means and gene effects are statistically different from zero at $P < 0.05$ and $P < 0.01$, respectively.

Table 6. Single-plant estimates of broad- (H) and narrow-sense (h^2) heritability ($\pm$ standard error) for Δ measured on parental, F_1 and segregating generations in two low $\times$ high Δ wheat crosses

Heritability	Cross	
	Quarrion/Genaro 81	Quarrion/Hartog
H	0.45 ± 0.09	0.41 ± 0.10
h^2	0.43 ± 0.07	0.29 ± 0.08

both additive and additive $\times$ additive epistatic effects were negative, indicating transmission of alleles for reduced Δ from the low Δ parent Quarrion. Estimates of broad- and narrow-sense heritability for Δ were moderate in size, differing significantly from zero when calculated on a single-plant basis (Table 6). Broad- and narrow-sense heritability estimates were similar for the Q/G cross, reflecting the lack of non-additive gene action. Differences in broad- and narrow-sense heritability were greater in the Q/H cross although this difference was not statistically significant.

Discussion

The three wheat genotypes Quarrion, Hartog and Genaro M81 ranked similarly across both studies. Quarrion consistently produced the smallest Δ, Hartog was intermediate whereas Genaro M81 is typically among the highest wheats for Δ (e.g. Condon et al., 1990, 1993). The relative ranking over the contrasting experiments and environments highlights the robustness and relatively small genotype $\times$ environment interaction for Δ when measured on leaf tissue sampled well before anthesis (Condon & Richards, 1992a; Rebetzke et al., 2002; Condon et al., 2004). Genetic differences among individual lines were attributable to nuclear and maternal genotypic factors. Evidence for nuclear genetic control of Δ was strong with large and repeatable genetic differences observed for parents and progeny across the different genetic designs. The F_1 progeny means agreed closely with midparent values in both half-diallel and generation means mating designs. In the generation means study, the F_1 and F_2 means were similar whereas backcross means were intermediate to the F_1 and recurrent parents. Thus backcrossing increased the frequency of alleles from the recurrent parent to change the direction of Δ toward the mean of the recurrent parent. Maternal effects were small and restricted to the Q/G cross only, and may

reflect cytoplasmic effects transmitted from the low Δ parent, Quarrion. Some of the genes associated with leaf photosynthesis are located in the chloroplast and are transmitted to progeny through the female parent.

The half-diallel and generation means mating designs indicated the importance of additive gene effects and to a lesser extent dominance and/or additive $\times$ additive epistasis in controlling expression of Δ. The negative sign on the additive gene effect indicated that additive alleles for lower Δ were largely transmitted for single and interacting loci from the low Δ Quarrion, and that the size of this effect was consistent across the Q/G and Q/H crosses. Evidence for simple additive gene action suggests that replacement and fixation of desirable alleles within a locus could be readily achieved in selection of lines with low Δ. However, in the case of epistasis, substitution for desired alleles relies on average effects of specific alleles at other interacting loci. Detection of epistasis and evidence of transgressive segregation suggested that variation for Δ was under oligo- or polygenic control. Thus it is conceivable that independent alleles at two or more loci could be pyramided into a single family for increased or decreased Δ.

The size of the GCA variance was c. five-fold larger than the SCA variance for Δ measured in the half-diallel. The strong correlation between GCA effects and parent *per se* performance indicates that a change in Δ can be readily achieved in progeny. Furthermore, the parental mean provides a good measure of predictability of hybrid performance, and provides a basis for designing crosses from which high or low Δ progeny can be recovered. A number of good, general combiners for low Δ were observed. The largest, negative GCA effect was associated with the drought-tolerant wheat variety, Quarrion. Quarrion is used in the CSIRO germplasm development program as a source of low Δ and high TE, and is a parent of two, low Δ-selected, commercial spring wheat varieties, 'Drysdale' and 'Rees'. Typically in this germplasm development program, large biparental and backcross populations are generated to recover one or few $F_{2:4}$ or $F_{3:4}$ progeny with Δ values approaching that of Quarrion. The need for larger population sizes reflects, in part, the need to accumulate several recessive alleles from Quarrion (e.g. Figure 1) to obtain low Δ genotypes. Delaying selection until lines are homozygous at all loci should increase the frequency of recessive homozygotes reducing the need for larger population sizes (Hanson, 1959). Selection for Δ is usually undertaken in one or few environments owing to small genotype $\times$ environment interaction

for leaf tissue sampled before flowering (Condon & Richards, 1992a; Rebetzke et al., 2002).

There was a large difference in the mean value of Δ measured in the two studies. Mean Δ in the half-diallel was 20.2‰, compared with 17.2‰ for the generation-means study. This difference could have been caused by several factors. Plants in the half-diallel were grown in large pots maintained to avoid soil water deficit, whereas plants in the generation means study were grown in the field. Despite the application of supplemental irrigation to the field-grown plants, the field soil may have been drier (Condon et al., 1992b) and/or harder (Masle & Farquhar, 1988), than in the pot study, to lower stomatal conductance for the field-grown plants and thereby reduce mean value of Δ. Tissues were sampled for Δ analysis from the generation-means study further into the spring than plants for the half-diallel. Average temperatures were warmer and vapor pressure deficit of the air likely to have been greater at the time of sampling in the generation means study, resulting in lower average stomatal conductance and lower Δ (Condon et al., 1992b). Also, plants from the generation-means study were more advanced phenologically. Stomatal conductance and Δ have been observed to decline with ontogeny (Condon et al., 1992a,b). Finally, tissue sampled for Δ from the generations-means study included a combination of leaf and stem material, whereas only leaf material was sampled from the half-diallel study. Values of Δ measured in stem material tend to be lower than in leaf material sampled at the same stage (Condon et al., 1992b), possibly due to higher lipid content in leaf blades.

There are very few reported estimates of gene action and combining ability for Δ in wheat. Genetic analysis of Δ has largely occurred in half-diallel studies of perennial species including alfalfa (*Medicago sativa* L.) (Johnson & Rumbaugh, 1995), and tree species maritime pine (*Pinus pinaster* Ait.) (Brendel et al., 2002) and black spruce (*Picea mariana* Mill.) (Johnsen et al., 1999). These studies reported consistently large and significant GCA, and small or non-significant SCA for Δ in F_1 progeny. A preponderance of GCA indicated large additive gene action and high narrow-sense heritability. In wheat, both additive and dominance gene action has been reported for Δ in progeny evaluated under irrigated conditions (Ehdaie & Waines, 1994). However, large genotype × environment interaction resulted in no genetic variance for Δ when measured under drought. Indeed, a lack of genetic variance for Δ in flag leaf tissue measured under severe soil water deficits highlights the importance

of measuring this trait under favourable conditions in order to maximise genetic variance and repeatability.

The evidence of predominantly additive gene action for Δ is consistent with the few studies reporting gene action for TE and/or its components, photosynthetic rate and leaf conductance. Both photosynthetic rate and leaf conductance are slow and difficult to measure, particularly for the large numbers of lines needed for genetic studies. Where reported, photosynthetic rate in wheat and tetraploid relatives is quantitatively inherited with largely additive genetic effects (Mahon, 1983; Carver et al., 1989). Simón (1994) also reported the importance of additive and epistatic-based gene action for photosynthetic rate measured on the 5th and flag leaves in two wheat crosses. Broad-sense heritability for photosynthetic rate was small in all studies.

Additive gene effects (as GCA) were large for TE in a 6 × 6 durum wheat diallel evaluated under well-watered and low moisture conditions in the glasshouse (Solomon & Labuschagne, 2004). Interaction of GCA and SCA with soil water treatment was small. Drought-based studies using populations derived from crosses between drought-resistant and susceptible bread wheat varieties indicate strongly additive genetic effects for instantaneous TE (as photosynthetic rate ÷ transpiration rate) and photosynthetic rate itself (Malik et al., 1999). Narrow-sense heritability was high for both traits. On the other hand, both broad – and narrow-sense heritability was small for leaf conductance measured in three high × low TE wheat crosses (Rebetzke et al., 2003). Delaying measurement until after 1200h increased genetic variance for leaf conductance, in turn increasing heritability. Gene action for leaf conductance in the three wheat crosses was largely additive with some evidence for additive-based epistasis. Carver et al. (1989) similarly reported small broad-sense heritability and largely additive gene effects for leaf conductance in hexaploid and tetraploid wheat crosses.

Genomic analysis has also been undertaken to investigate genetic control of Δ at the molecular level, and the potential for developing linked-molecular markers for use in marker-based, or assisted selection. Although not yet reported in wheat, QTL (Quantitative Trait Loci) have been identified for Δ in barley (Teulat et al., 2002), rice (Price et al., 2002), and cotton (Saranga et al., 2001). Genetic control was typically complex and associated with variation at many loci each accounting for a very small component of the total phenotypic variance. The potential for marker-based breeding for QTL of small genetic effect is somewhat limited given the higher narrow-sense heritabilities and

subsequent genetic gain for Δ with phenotypic selection.

Conclusion

Environmental differences between studies containing two different genetic designs produced large differences in mean Δ. Yet genotype ranking for Δ in lines common to both studies was the same as was the nature and importance of additive gene action in genetic control of Δ. A strong correlation for GCA and parent mean, as well as F_1 and midparent mean reflected largely additive gene action, and the predictive value of parent mean performance in developing low Δ progeny. Combining ability analysis also revealed that Quarrion was a good general combiner contributing recessive alleles to reduce Δ (high TE) in progeny. Evidence for dominance and additive-based epistasis highlights the need for some inbreeding and larger population sizes in order to recover families containing favourable inter-allelic combinations for low Δ. Inbreeding should also increase the frequency of recessive homozygotes to reduce minimum population sizes needed to recover low Δ progeny. Nevertheless, predominantly additive genetic control, large genetic variance and high heritability should ensure effective selection for Δ in populations segregating for Δ and high TE.

Acknowledgments

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