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ORIGINAL PAPER

# Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations

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**Abstract** Wheat productivity is commonly limited by a lack of water essential for growth. Carbon isotope discrimination (Δ), through its negative relationship with transpiration efficiency, has been used in selection of higher wheat yields in breeding for rainfed environments. The potential also exists for selection of increased Δ for improved adaptation to irrigated and high rainfall environments. Selection efficiency of Δ would be enhanced with a better understanding of its genetic control. Three wheat mapping populations (Cranbrook/Halberd, Sunco/Tasman and CD87/Katepwa) containing between 161 and 190 $F_1$-derived, doubled-haploid progeny were phenotyped for Δ and agronomic traits in 3–5 well-watered environments. The range for Δ was large among progeny (c. 1.2–2.3‰), contributing to moderate-to-high single environment ($h^2 = 0.37$–$0.91$) and line-mean (0.63–0.86) heritabilities. Transgressive segregation was large and genetic control complex with between 9 and 13 Δ quantitative trait loci (QTL) identified in each cross. The Δ QTL effects were commonly small, accounting for a modest 1–10% of the total additive genetic variance, while a number of chromosomal regions appeared in two or more populations (e.g. 1BL, 2BS, 3BS, 4AS, 4BS, 5AS, 7AS and 7BS). Some of the Δ genomic regions were associated with variation in heading date (e.g. 2DS, 4AS and 7AL) and/or plant height (e.g. 1BL, 4BS and 4DS) to confound genotypic associations between Δ and grain yield. As a group, high Δ progeny were significantly ($P < 0.10$–$0.01$) taller and flowered earlier but produced more biomass and grain yield in favorable environments. After removing the effect of height and heading date, strong genotypic correlations were observed for Δ and both yield and biomass across populations ($r_g = 0.29$–$0.57$, $P < 0.05$) as might be expected for the favorable experimental conditions. Thus selection for Δ appears beneficial in increasing grain yield and biomass in favorable environments. However, care must be taken to avoid confounding genotypic differences in Δ with stature and development time when selecting for improved biomass and yield especially in environments experiencing terminal droughts. Polygenic control and small size of individual QTL for Δ may reduce the potential for QTL in marker-assisted selection for improved yield of wheat.

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## Introduction

Breeding-era studies have demonstrated that much of the yield progress in wheat has been attributable to changes in carbon partitioning to grain expressed through a higher harvest index (Perry and D'Antuono 1989; Sayre et al. 1997). Genotypic increases in above-ground biomass also have potential to increase wheat yields under both well-watered and water-limited conditions (Fischer and Wood 1979). There has been little evidence for improvements in grain yield through greater biomass production except in parts of China (Zhou et al. 2007) and the UK

(Shearman et al. 2005). As selection for increased yields move closer to the theoretical limit for harvest index in wheat (Austin 1980) greater emphasis will be placed on increasing biomass through greater genetic variance from novel germplasm (e.g. chromosomal segments 1BL.1RS or 7DL.7Ag, Foulkes et al. 2007), and/or improved selection methods. Phenotypic selection for biomass is challenging as it is difficult to measure accurately, and has low heritability, especially in early, segregating generations of a breeding program (Sharma 1993; Rebetzke et al. 2002).

A number of component traits have been proposed for indirect selection of increased wheat biomass (e.g. Richards 2000). For example, high stomatal conductance and cooler canopy temperatures have been associated with greater biomass where water was non-limiting (Condon et al. 2007). However, profligate water use early may compromise later growth if water becomes limiting such as occurs with drought. 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 and 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 ($\Delta$) is associated with $c_i$ and therefore negatively correlated with TE (Condon et al. 1990). Use of $\Delta$ has potential in breeding programs as it both integrates TE over the period in which dry matter is assimilated and is simple to measure on large numbers of families (Condon et al. 2004). In a breeding program targeting adaptation to water-limited environments, indirect selection for high biomass and yield via low $\Delta$ can be more efficient than direct selection of either production trait in early generations (Rebetzke et al. 2002).

Evaluation of $BC_2$-derived, sister-lines showed $\Delta$ to be negatively correlated with aerial biomass and yield for wheat evaluated in water-limited, rain-fed environments (Rebetzke et al. 2002). In other instances the relationship between $\Delta$ and grain yield of temperate cereals has been positive. In wheat, genotypic increases in $\Delta$ were associated with increases in aerial biomass and yield in favorable environments (e.g. Condon et al. 1987, 2004; Morgan et al. 1993; Fischer et al. 1998). High $\Delta$ has been associated with higher leaf conductance, increased water use and growth (Fischer et al. 1998; Condon et al. 2004). Hence positive relationships for $\Delta$ and yield have been obtained in favorable irrigated environments where water supply was not a major constraint to yield (e.g. Fischer et al. 1998), or in Mediterranean environments where soil water up to anthesis was plentiful (Araus et al. 2003). Thus, the opportunity exists to select for high $\Delta$ where water for crop growth is sufficiently abundant.

An understanding of inheritance is essential in development of strategies aimed at efficient selection of $\Delta$. Repeatable genotypic variation has been reported in wheat for TE (Condon et al. 1993; Malik et al. 1999; Solomon and Labuschagne 2004) and $\Delta$ (Condon and Richards 1992; Rebetzke et al. 2002, 2006). These reports emphasize that broad- and narrow-sense heritability of $\Delta$ is high when expressed on a single-plot or entry-mean basis (Condon and Richards 1992; Rebetzke et al. 2002). Further, analysis of mating designs employing progeny from either $F_1$ or segregating generations has shown $\Delta$ to be under strong additive genetic control with little evidence for non-additive gene action (Rebetzke et al. 2006). Together, these results indicate that family selection for altered $\Delta$ in early generations will likely produce correlated changes in $\Delta$ among inbred lines.

Genomic analysis of underlying quantitative trait loci (QTL) offers the potential to extend quantitative genetic analysis of phenotypes to understanding of genetic control at the molecular and thus genotypic level. Repeatable regions may then be targeted in the development of linked molecular markers for use in marker-assisted selection (MAS). QTL for $\Delta$ have been reported across a range of species including *Arabidopsis* (Hausmann et al. 2005), barley (*Hordeum vulgare*) (Forster et al. 2004), rice (*Orzya indica*) (Laza et al. 2006) and cotton (*Gossypium hirsutum*) (Saranga et al. 2001). No QTL have yet been described for $\Delta$ in wheat. Robustness is vital in establishing value of QTL in trait dissection and/or use in MAS (Wang et al. 2007). Few studies have established the repeatability of genomic regions for $\Delta$ across populations and environments. Hence there is a critical need for QTL studies to be undertaken across multiple environments, and validated either across populations, or in selection response studies. This study reports on multi-environment experiments aimed at phenotyping and subsequent QTL identification for $\Delta$ across three wheat populations.

## Materials and methods

### Populations and genotypes

Three populations containing between 161 and 190 doubled-haploid (DH) lines were derived from crosses between spring wheats Cranbrook and Halberd (hereafter C/H), Sunco and Tasman (S/T), and CD87 and Katepwa (Cd/K). Cranbrook, Halberd, Sunco and Tasman are broadly adapted, high-yielding Australian commercial wheat varieties; CD87 was a broadly adapted wheat breeding line and Katepwa was of Canadian origin. Coancestries among

parents varied from a low 0.06 for C/H, to 0.15 and 0.36 for Cd/K and S/T, respectively. Across populations, the S/T and Cd/K populations were genetically related through WW15 as a parent in the pedigrees of Sunco, Tasman and CD87. Development of each population is described in detail in Kammholz et al. (2001).

## Experimental design and sampling

Parents and DH progeny from the C/H, S/T and Cd/K populations were sown with the low Δ control Quarrion, into 6-m long, 5-row, bordered plots (plot area of c. 5.4 $m^2$) at Ginninderra Experiment Station (GES), Australian Capital Territory in 2002, 2003 and 2004. All populations were sown adjacent to each other except Cd/K in 2004 where sowing was delayed 10 weeks (and genotypes sown as single rows) owing to a lack of space. In addition to these sowings, additional sowings of the C/H population were made late in 2003 at GES and in 2004 at Gundibindyal NSW. Experiments were multiple-augmented designs with one or two replicates, and parents and Quarrion each replicated ten times. Crops were sown after canola or lucerne break-crops and then managed with adequate nutrition and spraying of pesticides to control weeds and leaf diseases. Adequate irrigation was supplied at GES to avoid water-stress while crops at Gundibindyal were reliant on rainfall.

Leaves were sampled from 10 to 20 plants at peak-tillering (Z31, Zadoks et al. 1974) at GES in 2002, 2003, and 2004. Additional sampling of leaves was undertaken for C/H in a late-sown experiment at GES in 2003, and whole stems were sampled at heading for C/H at Gundibindyal in 2004. Harvested plant tissue was dried at 70°C for 3 days before being ground to pass a 0.5 mm sieve. The $^{13}C$:$^{12}C$ composition was determined for each sample by ratio mass spectrometry using a Micromass Isochrom mass spectrometer. Carbon isotope discrimination was calculated assuming the $^{13}C$:$^{12}C$ composition of $CO_2$ in air equals −8‰. Use of repeated standards allowed accuracy of the Δ measurements on the mass spectrometer to be determined at ±0.1‰. Development was monitored regularly and heading date recorded when 50% of ears in a plot had flowered. Plant height determined at maturity as the distance from the soil surface to the top of the spike (awns excluded) of the tallest culms for each plot. Plots were subsampled at maturity with 0.3 $m^2$ quadrats. Whole dried stems were hand-cut at ground level and placed into bags. Samples were then weighed, spikes counted and grain threshed. Plots were end-trimmed to approximately 5 m and the outside border rows removed before machine harvesting to obtain plot yields. Harvest index was calculated as the ratio of grain to total culm weight of the subsamples, and aerial biomass then calculated as plot grain yield/harvest index. Grain weight was determined for a 200-grain sample from each plot, and grain number ($m^{-2}$) calculated as grain yield/grain weight.

## Statistical analyses

Variance and covariance components for genotype and genotype × environment interaction were estimated assuming environments were fixed and row, column and genotypes within populations random effects. Variance components and their SEs were obtained following analysis by the method of restricted maximum likelihood using mixed linear models in the SAS procedure MIXED (Littell et al. 1996). Narrow-sense heritability ($h^2$) was then calculated for each population on a genotype-mean basis assuming the covariance among $F_1$-derived, doubled-haploids in each population equated to $1\sigma^2_A + 1\sigma^2_{AA}$ (where, $\sigma^2_A$ and $\sigma^2_{AA}$, refers to additive and additive × additive epistatic genetic variances, respectively). The putative numbers of genes varying for Δ in each population and environment was estimated after Mather and Jinks (1982) as $(0.5D)^2/\sigma^2_A$, where $D$ was the range among progeny and $\sigma^2_A$ the additive genetic variance.

## QTL mapping

The genetic maps described for each population in Lehmensiek et al. (2005) and subsequently updated (A. Lehmensiek, personal communication) contain between 400 and 800 microsatellite, morphological, biochemical and DArT markers. QTL analysis was done using spatially adjusted BLUEs for each environment and mixed linear composite interval mapping was undertaken in QTLNetwork 2.0 (Yang et al. 2005). Composite interval analysis was undertaken using forward–backward stepwise, multiple linear regression with a probability into and out of the model of 0.05 and window size set at 10 cM. Significant thresholds for QTL detection were calculated for each dataset using 1,000 permutations (Churchill and Doerge 1994) and a genome-wide error rate (α) of 0.10 (suggestive) and 0.05 (significant). The resulting genetic model incorporated significant main additive and additive × additive epistatic genetic effects and their interactions with environment. Locations of genetic effects of individual QTL were identified from maps drawn using MapChart 2.1 (Voorrips 2002), and 95% confidence intervals for each QTL location were obtained through jack-knifing (Yang et al. 2005).

## Correlated genetic effects

Genetic influence of Δ on agronomic traits was assessed via correlated response following retrospective, divergent

selection. Selection intensity was set at 20% (c. 30 lines) and differences in the means of selected low- and high-Δ sub-populations determined statistically. The statistical model used for each analysis was: $Y_{ijk} = \mu + E_i + S_j + L(S)_{j(k)} + SE_{ij} + \varepsilon_{ijk}$, where $\mu$ = the overall mean; $E_i$ = effect of environment $i$; $S_j$ = effect of selection $j$; $L(S)_{j(k)}$ = effect of line $k$ within selection $j$; $SE_{ij}$ = the interaction of selection $j$ with environment $i$; and $\varepsilon_{ijk}$ = the residual variation. Statistical significance of among-selection differences was tested using the selection × environment variance (Rawlings 1988).Genotypic correlations and their approximate SEs were also estimated between Δ and agronomic traits for all lines after Falconer and Mackay (1996). Variation in plant height and heading date were removed through covariance analysis.

Assessment of correlated Δ QTL effects with agronomic traits was undertaken using the nearest significant Δ-linked marker and fitting a mixed linear model: $Y_{ijk} = \mu + E_i + M_j + L(M)_{j(k)} + ME_{ij} + L(M)E_{k(j)i} + \varepsilon_{ijk}$, where $\mu$ = the overall mean; $E_i$ = effect of environment $i$; $M_j$ = effect of marker $j$; $L(M)_{k(j)}$ = effect of line $k$ within marker $j$; $ME_{ij}$ = the interaction of marker $j$ with environment $i$; $L(M)E_{k(j)i}$ = interaction of environment $i$ with line $k$ within marker $j$ and $\varepsilon_{ijk}$ = the residual variation effect. Environment, QTL (as the nearest linked marker), selection, and their interactions were fixed, and line nested within marker or selection, and row and column factors were random effects in SAS procedure MIXED. Statistical significance of individual marker effects was tested using the line within marker × environment variance (Rawlings 1988).

## Results

### Characterisation of phenotypic effects

#### *Environments and sampling for Δ*

Large differences were observed for Δ across environments for all three populations (Tables 1, 2). These environmental effects were evident as changes in population, parental and control (cv. Quarrion) Δ means across years and sites. For example, the low-Δ control, Quarrion, varied c. 3.3‰ from 2002 through to 2004 in both the C/H and S/T populations (Table 1). For the C/H population, Δ was lowest for the GES 2003 late-sown and Gundibindyal sowings, and highest for the GES 2004 sowing. Across environments, Δ and vapor pressure deficit (VPD) were negatively albeit weakly correlated ($r = 0.50$, $P > 0.05$; Table 2) so that increasing aerial aridity was associated with reductions in Δ. The sampled range of sites and years provided a contrasting set of conditions over which agronomic performance for the different populations could be assessed. For example, in the C/H population, total biomass, grain yield, and grain number were largest for the irrigated GES environments and smallest for the rainfed Gundibindyal site (Table 2). Over all environments, relationships with total water (rain + irrigation) were strongest for grain yield ($r = 0.97$, $P < 0.01$) and smallest for grain weight ($r = 0.84$, $P < 0.10$). The importance of water for growth and productivity was mirrored in significant, positive associations of total water (rainfall + irrigation) with total biomass, harvest index and numbers of grain across environments (Table 2).

#### *Populations and genotypes*

Populations differed in mean Δ, with the S/T population producing a smaller mean Δ than both the low Δ check Quarrion and the C/H or Cd/K populations (Table 1; Fig. 1). An exception was the 2004 Cd/K population which was sown very late under conditions of high VPD (data not shown). The smaller mean of the S/T population mirrored the smaller mean values of Sunco and Tasman relative to parents of the C/H and Cd/K populations (Table 1). Indeed, over all environments and populations, mid-parent and progeny-mean Δ were linearly associated ($Y_{\text{Prog}} = -0.47 + 1.02\ X_{\text{MP}}$, $r^2 = 0.98$, $P < 0.01$). Hence mid-parent Δ was a good predictor of mean progeny Δ, highlighting the importance of additive gene effects and the subsequent utility of the DH (homozygous) mapping populations for genetic analysis of Δ. However, the larger progeny mean for the Cd/K population (Table 1) indicates some potential for some non-additive gene action in this cross.

Parents were significantly ($P < 0.05$) different for Δ in the C/H and S/T populations but were similar for the Cd/K population where measured (Table 1). Differences between parents were reasonably consistent across years, a notable exception being CD87 and Katepwa in GES 2002, and Halberd and Cranbrook in Gundibindyal 2004 which did not differ. The low Δ control Quarrion was consistently low for Δ across all experiments, and was among the lowest 30% of all tested lines for this trait (Table 1). The consistent ranking of both parents and Quarrion across environments demonstrated the high repeatability of Δ in vegetative tissue.

Progeny within each population were significantly ($P < 0.01$) different for Δ in all environments (Table 1). Distributions of progeny means were typically gaussian with the exception of the Cd/K population in 2004, where progeny were skewed toward high Δ (Fig. 1). Progeny extremes for Δ commonly exceeded either parent, indicating transgressive segregation at multiple loci for Δ. Since narrow-sense heritability was commonly high for Δ

**Table 1** Carbon isotope discrimination (‰) and estimates of narrow-sense heritability and number of genes for DH progeny, parents and control genotype for three DH mapping populations grown under irrigated conditions at Ginninderra Experiment Station, ACT in 2002, 2003 and 2004

| Entry | | Cranbrook/Halberd | | | | | Sunco/Tasman | | | CD87/Katepwa | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2002 | 2003 | 2004 | 2003L | 2004Gu | 2002 | 2003 | 2004 | 2002 | 2003 | 2004 |
| Progeny | Minimum | 17.95 | 19.09 | 20.62 | 17.30 | 17.21 | 17.09 | 17.78 | 19.58 | 17.86 | 18.92 | 18.75 |
| | Maximum | 19.65** | 21.05** | 22.37** | 18.71** | 18.39** | 18.80** | 19.36** | 21.29** | 19.44** | 20.09** | 21.16** |
| | lsd[a] | 0.65 | 0.45 | 0.39 | 0.79 | 0.67 | 0.60 | 0.42 | 0.41 | 0.64 | 0.40 | 0.40 |
| Mean | | 18.85 | 20.31 | 21.57 | 18.05 | 17.78 | 17.87 | 18.67 | 20.46 | 18.53 | 19.52 | 20.24 |
| | $h^2$ | 0.69 | 0.76 | 0.91 | 0.37 | 0.43 | 0.61 | 0.67 | 0.74 | 0.62 | 0.73 | 0.54 |
| No. of genes | | 4.8 | 10.8 | 7.3 | 5.4 | 3.4 | 12.2 | 10.4 | 7.3 | 6.2 | 4.7 | 7.1 |
| Parents | | 18.39 (C) | 20.08 | 21.11 | 17.97 | 18.28 | 17.98 (S) | 18.85 | 20.55 | 18.73 (Cd) | 19.28 | 20.43 |
| | | 19.32 (H) | 20.72 | 21.94 | 18.24 | 18.21 | 17.60 (T) | 18.41 | 20.39 | 18.78 (K) | – | 20.89 |
| Control | Quarrion | 17.85 | 19.27 | 20.62 | 17.34 | 17.25 | 17.96 | 19.02 | 20.60 | 17.81 | 19.00 | 18.99 |
| | lsd[b] | 0.20 | 0.15 | 0.31 | 0.30 | 0.33 | 0.27 | 0.15 | 0.15 | 0.17 | 0.11 | 0.19 |

The C/H population was also evaluated with late-sowing at GES in 2003 (2003L) and under rain-fed conditions at Gundibindyal in 2004 (2004Gu)

** Indicate differences between DH progeny are statistically significant at $P = 0.01$

[a] Average SE of a difference for comparisons among DH progeny

[b] Average SE of a difference for comparisons among controls

**Table 2** Average trait and climatic data from sowing to heading for Ginninderra Experiment Station (GES) and Gundibindyal sites in each year for the Cranbrook/Halberd population

| Site/year | Sowing date | Mean min. (°C) | Mean max. (°C) | Total rainfall (mm) | Daily VPD (kPa) | Solar radiation (MJ $m^{-2}$) | $\Delta$[a] (‰) | Grain yield (t $ha^{-1}$) | Total biomass (t $ha^{-1}$) | Grain weight (mg) | Grain number ($m^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GES[b] | | | | | | | | | | | |
| 2002 | 6 June | 1.9 | 15.6 | 150[a] | 0.94 | 2,086 | 18.85 | 5.52 | 14.2 | 38.7 | 14,221 |
| 2003 (early) | 17 June | 3.0 | 14.5 | 231 | 0.71 | 2,059 | 20.31 | 6.71 | 16.1 | 42.2 | 15,976 |
| 2003 (late) | 24 Sept | 9.8 | 24.0 | 173 | 2.01 | 1,392 | 18.05 | –[c] | – | – | – |
| 2004 | 7 June | 2.9 | 15.3 | 191 | 0.79 | 1,895 | 21.57 | 5.82 | 14.6 | 38.3 | 15,180 |
| Gundibindyal | | | | | | | | | | | |
| 2004 | 2 June | 4.2 | 15.9 | 205 | 0.81 | 1,770 | 17.78 | 2.29 | 6.0 | 39.7 | 5,760 |

[a] All $\Delta$ values are for Z31 leaf samples except Gundibindyal 2004 where whole shoots were sampled at heading

[b] Ginninderra received up to six supplemental 25 mm irrigations throughout the pre- and post-heading period in each year

[c] Not measured

measured on Z31-sampled leaves (Table 1), these distributions largely reflected genetic rather than environmental variance. For the C/H population, the range in progeny values was greatest for high mean $\Delta$ environments GES 2003 early-sown and GES 2004, and smallest for low $\Delta$ environments Gundibindyal 2004 and GES 2003 late-sown. In turn, heritabilities were lower and SEs of differences larger for $\Delta$ measured on Z31-sampled leaves growing under conditions of high VPD (i.e. GES 2003 late-sown) and developmentally later vegetative tissue (i.e. whole stems sampled at Z65 at Gundibindyal in 2004). This reduced the correlation of phenotype with genotype for $\Delta$ to reduce confidence in the range and genetic variance for $\Delta$ (Table 1). The large range for $\Delta$ among progeny contributed toward significant ($P < 0.01$) genetic variance for this trait in all populations in each environment. Extension to analysis across all environments (Table 3) showed that genotypic variances were large relative to genotype × environment interaction variances, to increase narrow-sense heritabilities in all three populations. The range in progeny $\Delta$ was large and similar (c. 1.3 per mil) for all three populations (Table 4).

Genotypic variation was also large and statistically significant ($P < 0.01$) for a range of agronomic traits

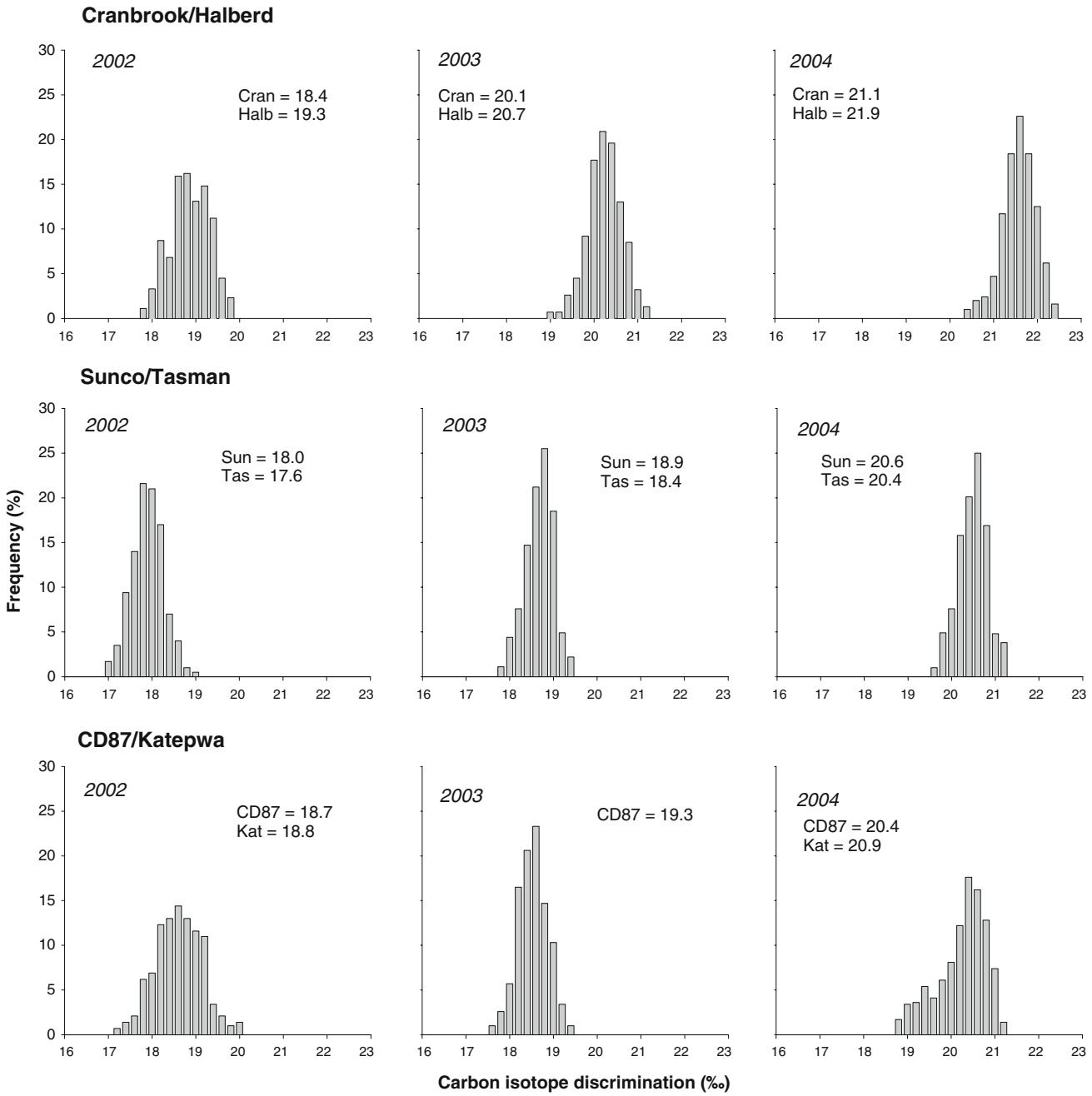


**Fig. 1** Frequency distributions for carbon isotope discrimination (Δ) means measured on random DH lines from the Cranbrook/Halberd, Sunco/Tasman, and CD87/Katepwa mapping populations grown at GES. Parental means are indicated for each environment

measured in each population. Table 4 summarises the range among DH progeny in each population for different traits averaged across environments. Parental means are also included for comparison and highlight transgressive segregation for most traits measured. The S/T population was later-heading and showed the smallest range in progeny heading date among populations (Table 4). The largest range in plant height was for the S/T population segregating for both *Rht-B1b* and *Rht-D1b* dwarfing genes. The C/H and Cd/K populations were also wide-ranging for height despite the single *Rht-B1b* dwarfing gene segregating in both populations. Genotype × environment interaction was commonly small for days to heading and plant height, and therefore heritability was high (data not shown). Grain yield, total biomass and harvest index were wide-ranging in all three populations (Table 4) although large genotype × environment interaction reduced heritability for grain yield and biomass (data not shown).

**Table 3** Genotype ($\sigma^2_G$) and genotype × environment interaction ($\sigma^2_{GE}$) variances (±SE), and narrow-sense heritability estimates on a genotype-mean basis for carbon isotope discrimination (‰) measured on random, DH progeny from three populations (Cranbrook/Halberd, C/H; Sunco/Tasman, S/T; and CD87/Katepwa, Cd/K) grown under irrigated and rainfed conditions in 2002, 2003 and 2004

| Parameter | C/H | S/T | Cd/K |
|---|---|---|---|
| $\sigma^2_G$ | 0.066 ± 0.009** | 0.049 ± 0.008** | 0.081 ± 0.015** |
| $\sigma^2_{GE}$ | 0.012 ± 0.005* | 0.007 ± 0.012 ns | 0.015 ± 0.019 ns |
| $h^2$ | 0.86 | 0.76 | 0.77 |

*,** Indicate variance components are statistically different from zero at $P = 0.05$ and 0.01, respectively

*ns* Indicates variance components are not statistically different from zero at $P > 0.05$

## Correlated effects of Δ on agronomic traits

### *Retrospective selection*

Retrospective selection of the 20% highest and lowest Δ DH lines into divergent Δ groups was undertaken to assess correlated changes in agronomic performance with selection for Δ (Table 5). Mean differences in Δ for the high and low Δ-selected groups were 0.72, 0.78 and 1.08‰ for the C/H, S/T and Cd/K populations, respectively. Associated changes in plant height and development were commonly small but statistically significant ($P < 0.01$). Low Δ-selected groups were of shorter stature and later-heading than their high Δ-selected counterparts. Selection for high Δ was associated with greater aerial biomass. Despite slightly smaller harvest index, greater biomass in the high Δ group was associated with larger grain yields, particularly in the Cd/K population. Increased grain yield in Cd/K reflected small but positive increases in grain weight and number.

### *Genetic correlations*

Across all lines in each population, genotypic increases in plant height and delays in heading date were associated with reductions in grain number and harvest index to reduce grain yield (Table 6). Later heading was associated with greater biomass particularly in the wider-ranging heading date populations C/H and Cd/K. Genotypic increases in Δ were commonly associated with increased plant height and earlier heading (Table 7a). The relationship with height was strongest for the S/T population which varies for two major dwarfing genes (*Rht-B1b* and *Rht-D1b*), and strongest with heading date for the C/H population. Increased Δ was associated with increases in grain yield and aerial biomass in the S/T and Cd/K populations but reduced harvest index in all three populations. Increases in yield were largely due to greater grain weight, although increased Δ was associated with greater grain number in the S/T population.

Efforts were made to remove the correlated effects of plant height and development on Δ and agronomic traits through covariance analysis (Table 7b). The analysis showed a small reduction in the genetic variance for Δ and consequently small change in line-mean heritability

**Table 4** Range among DH progeny for different characteristics measured under irrigated conditions at Ginninderra Experiment Station, ACT in 2002, 2003 and 2004

| Population | Parameter | Δ (‰) | Days to heading (days) | Plant height (cm) | Grain yield (t ha$^{-1}$) | Aerial biomass (t ha$^{-1}$) | Harvest index |
|---|---|---|---|---|---|---|---|
| C/H | Minimum | 19.60 | 136 | 74 | 4.29 | 10.9 | 0.30 |
| | Maximum | 20.94 | 146 | 121 | 8.11 | 18.9 | 0.49 |
| | $P_1$, $P_2$ | 19.88, 20.65 | 140, 141 | 85, 100 | 6.61, 6.43 | 14.4, 15.8 | 0.46, 0.41 |
| | LSD[a] | 0.31 (0.28) | 2 (2) | 9 (6) | 1.1 (0.9) | 2.2 (1.9) | 0.04 (0.03) |
| S/T | Minimum | 18.43 | 144 | 48 | 3.35 | 10.9 | 0.28 |
| | Maximum | 19.72 | 149 | 109 | 8.28 | 18.5 | 0.52 |
| | $P_1$, $P_2$ | 19.14, 18.82 | 147, 148 | 84, 82 | 6.67, 6.76 | 14.9, 15.2 | 0.44, 0.45 |
| | LSD | 0.38 (0.28) | 2 (2) | 8 (7) | 1.4 (1.1) | 3.1 (2.9) | 0.04 (0.03) |
| Cd/K[b] | Minimum | 18.72 | 135 | 80 | 3.53 | 9.2 | 0.30 |
| | Maximum | 20.25 | 145 | 134 | 7.22 | 20.4 | 0.47 |
| | $P_1$, $P_2$ | 19.59, –[c] | 141, – | 84, – | 6.16, – | 14.3, – | 0.43, – |
| | LSD | 0.54 (0.38) | 3 (2) | 9 (9) | 1.8 (1.6) | 4.5 (4.4) | 0.05 (0.03) |

Populations are Cranbrook/Halberd (C/H); Sunco/Tasman (S/T); and CD87/Katepwa (Cd/K). Parent 1 ($P_1$) and parent 2 ($P_2$) means are also included for comparison

[a] Least significant difference ($P = 0.05$) for comparisons between progeny lines. Values in parenthesis are LSDs for comparisons with each parent

[b] No agronomic data for Cd/K in 2004

[c] Parent Katepwa excluded as not measured in all environments

**Table 5** Correlated change in a range of agronomic characteristics with retrospective, divergent selection for low and high carbon isotope discrimination (Δ) in three wheat mapping populations (Cranbrook/Halberd (C/H); Sunco/Tasman (S/T); and CD87/Katepwa (Cd/K)) evaluated under irrigation at Ginninderra Experiment Station, ACT in 2002, 2003 and 2004

| Population | Selected group | Δ (‰) | Plant height (cm) | Days to heading (d) | Grain yield (t ha$^{-1}$) | Aerial biomass (t ha$^{-1}$) | Harvest index | Grain weight (mg) | Number grains (m$^{-2}$) | No. of spikes (m$^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| C/H | Low | 19.87 | 92 | 142 | 6.17 | 14.9 | 0.42 | 39.6 | 16,051 | 480 |
| | High | 20.59 | 95 | 139 | 6.27 | 15.3 | 0.41 | 41.4 | 15,492 | 491 |
| | *F* | ** | ** | ** | ns | *** | ns | * | ns | ns |
| S/T | Low | 18.60 | 73 | 147 | 6.19 | 14.5 | 0.43 | 38.6 | 16,445 | 567 |
| | High | 19.38 | 85 | 146 | 6.38 | 15.2 | 0.41 | 38.4 | 16,130 | 618 |
| | *F* | ** | ** | * | *** | * | * | ns | ns | *** |
| Cd/K | Low | 18.54 | 99 | 141 | 5.50 | 14.1 | 0.39 | 37.2 | 14,885 | 515 |
| | High | 19.62 | 107 | 139 | 5.91 | 16.1 | 0.37 | 38.2 | 15,601 | 520 |
| | *F* | ** | ** | * | * | ** | *** | * | *** | ns |

Evaluation of the Cd/K population was undertaken in 2002 and 2003 only

*,**, *** Indicate difference between low and high carbon isotope discrimination selected groups are statistically significant at $P$ =0.05, 0.01 and 0.10, respectively

*ns* Indicates difference between low and high carbon isotope discrimination selected groups is not statistically significant at $P > 0.10$

**Table 6** Genetic correlations for (a) plant height and (b) days to heading with grain yield, total biomass and harvest index in three populations (Cranbrook/Halberd (C/H); Sunco/Tasman (S/T); and CD87/Katepwa (Cd/K)) evaluated under irrigation at Ginninderra Experiment Station, ACT in 2002, 2003 and 2004 (Cd/K in 2002 and 2003 only)

| Trait | Population | Grain yield | Aerial biomass | Harvest index | Number of grains |
|---|---|---|---|---|---|
| (a) Plant height | C/H | −0.80** | 0.10 | −0.75** | −0.51** |
| | S/T | −0.38** | −0.11 | −0.42** | −0.61** |
| | Cd/K | −0.70** | −0.49** | −0.66** | −0.55** |
| (b) Days to heading | C/H | −0.33* | 0.78** | −0.81** | −0.31* |
| | S/T | −0.49** | 0.21* | −0.61** | −0.24* |
| | Cd/K | −0.29* | 0.75** | −0.59** | −0.77** |

*,** Indicate genetic correlation is statistically different from zero at $P = 0.05$ and 0.01, respectively

**Table 7** Genotypic correlations for carbon isotope discrimination and a range of agronomic characteristics in three populations [Cranbrook/Halberd (C/H); Sunco/Tasman (S/T); and CD87/Katepwa (Cd/K)] evaluated under irrigation at Ginninderra Experiment Station, ACT in 2002, 2003 and 2004 (Cd/K in 2002 and 2003 only)

| Analysis | Population | Plant height | Days to heading | Grain yield | Aerial biomass | Harvest index | Grain weight | Number of grain | $\sigma^2_G \pm$ se | $h^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| (a) All | C/H | 0.17* | −0.21* | 0.09 | −0.11 | −0.14* | 0.18* | −0.13* | 0.066 ± 0.009 | 0.86 |
| | S/T | 0.33* | −0.11* | 0.32* | 0.36** | −0.17* | 0.24* | 0.35** | 0.049 ± 0.008 | 0.76 |
| | Cd/K | 0.28* | −0.14* | 0.13* | 0.52** | −0.28* | 0.16* | −0.07 | 0.081 ± 0.015 | 0.77 |
| (b) Covariance | C/H | – | – | 0.33* | 0.39** | −0.05 | −0.07 | 0.23* | 0.050 ± 0.009 | 0.73 |
| | S/T | – | – | 0.29* | 0.25* | 0.14* | −0.17* | 0.33* | 0.046 ± 0.007 | 0.71 |
| | Cd/K | – | – | 0.57** | 0.56** | 0.20* | 0.04 | 0.47** | 0.068 ± 0.014 | 0.66 |

Correlations are given unadjusted for genotypic differences in height and flowering (a), and after fitting plant height and heading date as covariables (b)

Genotypic variance and line-mean heritability for Δ are also given for each analysis

*,**, *** Indicate genetic correlation is statistically different from zero at $P = 0.05$, 0.01 and 0.10, respectively

particularly in the C/H and Cd/K populations. Resulting relationships between Δ and both biomass and yield were stronger, largely through stronger relationships of Δ with grain number. Relationships for Δ with harvest index were close to zero or positive across populations and were variable with grain weight (Table 6).

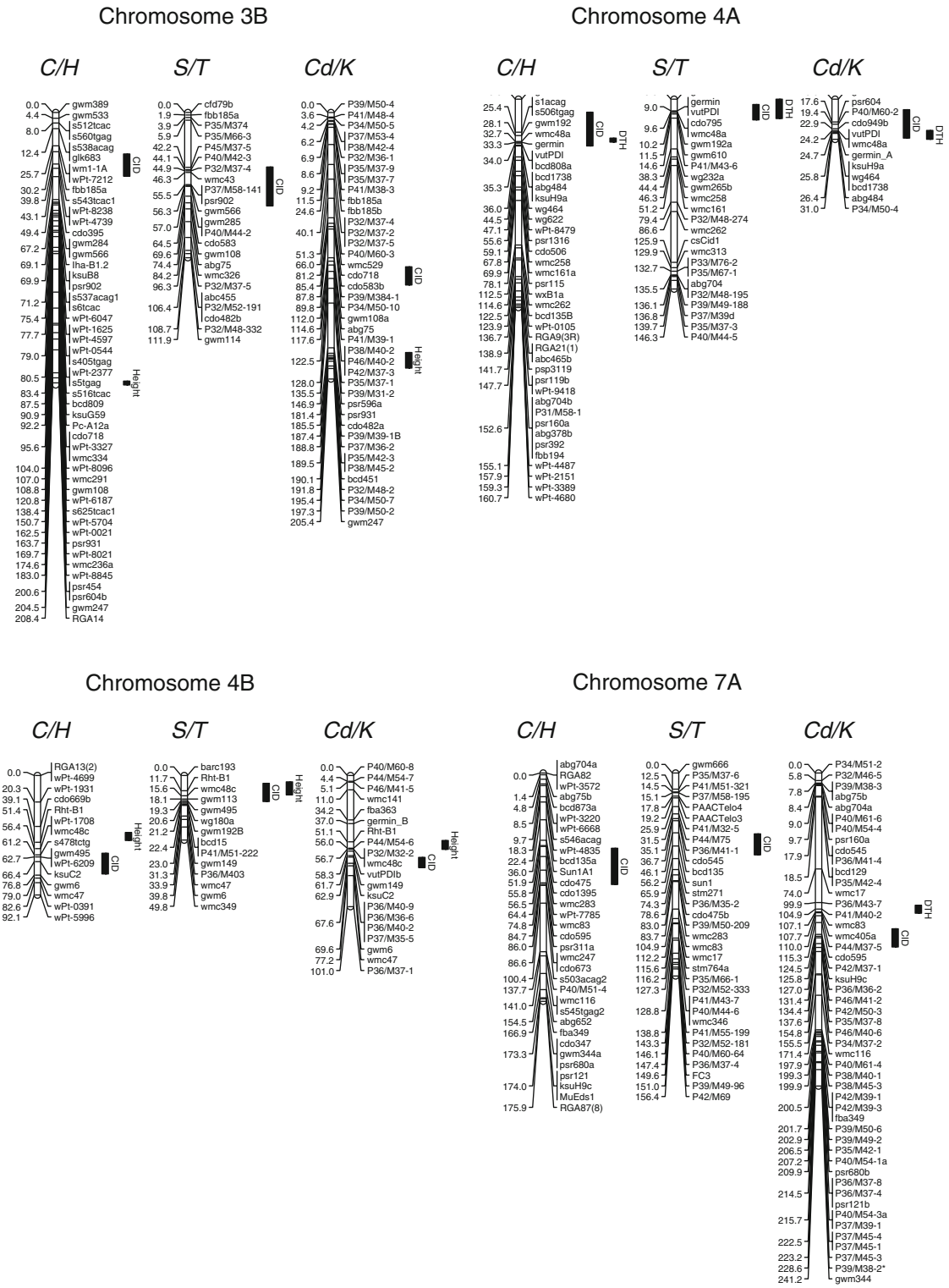


**Fig. 2** Chromosomal locations of QTL for carbon isotope discrimination (*CID*) measured on random DH lines from the Cranbrook/Halberd (*C/H*), Sunco/Tasman (*S/T*), and CD87/Katepwa (*Cd/K*) mapping populations for different chromosomes: **a** 3B, **b** 4A, **c** 4B, and **d** 7A. QTL for days to heading (*DTH*) and plant height (*height*) are also indicated. QTL are indicated to the right of the linkage group and the highlighted bar represents the 95% confidence interval for the QTL

QTL analysis

Comprehensive molecular maps with good chromosomal coverage were available for all three populations (Lehmensiek et al. 2005). Plant height QTL of major genetic effect were identified on chromosomes 4B and 4D and co-located with known *Rht-B1b* and *Rht-D1b* genes in all three populations (Table 8; Fig. 2). Similarly, a large heading date QTL was located on chromosome 2DS in the C/H populations consistent with the location of alleles at the *Ppd1* locus near marker *gwm261* (Table 8).

From the single environment analysis, the estimated numbers of genes affecting Δ ranged from 3 to 12 depending on population and environment (Table 1). Similarly, a large number of significant QTL were identified for Δ in each population in each environment (data not shown). These QTL varied in size and in the proportion of phenotypic variance accounted for. When estimated from the multi-environment analysis, the number of genes affecting Δ was high in each population ($n = 9.6$, 12.4 and 7.3 for C/H, S/T and Cd/K, respectively). Extension of QTL analyses to a one-step, multi-environment model identified a number of QTL in each of the three populations (Table 8). The identified Δ QTL were all of small genetic effect and individually accounted for less than 10% of the additive genetic variance. Few of the QTL showed a significant interaction with environment. Alleles for increased Δ were contributed by either parent which, together with the identification of multiple QTL, supported the transgressive segregation observed for Δ in all three populations (Fig. 1). Epistatic (additive × additive) genetic effects were small, accounting for little of the total genotypic variance (data not shown).

Several Δ QTL seemingly co-located across populations (Table 8; Fig. 2). There were a number of these QTL identified in two or more populations, for which genetic effects were largely independent of changes in plant height and heading date. These QTL were located on chromosomes 2BS, 6DL, 7AS and 7BS (Table 8; Fig. 2). On the other hand, there were Δ QTL associated with genotypic variation in plant stature and development. For example, Δ QTL mapped to alleles at the *Rht-B1* (4BS) and *Rht-D1* (4DS) dwarfing loci, and at loci associated with variation in heading date (e.g. 4AS). Increased Δ at these QTL was commonly associated with taller plant height and fewer days to heading (Table 8) consistent with the genetic correlation of Δ with these agronomic traits (Table 6). For example, the low Δ allele on chromosome 4AS was associated with later heading date in all three populations (Table 8). In the S/T population segregating for alleles at both *Rht-B1* and *Rht-D1* loci, mean Δ decreased linearly ($r = -0.98$, $P < 0.05$) with increasing frequency of dwarfing alleles (from 0 to 4). The Δ means in the S/T populations were 19.11‰ for the tall, 19.01 for the *Rht-B1b* semi-dwarf, 18.98 for the *Rht-D1b* semi-dwarf and 18.92‰ for the doubled-dwarf genotypic classes, respectively. Allelic effects at height loci were additive with no evidence of any epistatic interaction among loci ($P = 0.73$ ns).

Other Δ QTL were identified specific to populations. Some of these were associated with variation in plant height (e.g. 1BL and 2AS in C/H) while others covaried with variation in heading date (e.g. 2AS in C/H, 1DL in S/T and 6BS in Cd/K). Importantly, the nature of these Δ effects were not always consistent with changes in plant height or development. For example, the low Δ allele for the QTL on 1BL was associated with a reduction in plant height in the C/H population, a neutral height effect in the S/T population, and increased height in the Cd/K population (Table 8). Despite the variation in plant height and heading date in all three populations, a number of Δ QTL were identified that were independent of height and development effects (e.g. 5AS and 5AL in C/H).

Correlated genetic effects at individual Δ QTL were ascertained for a range of agronomic traits including aerial biomass, grain yield and grain number (Table 8). Because observed Δ effects were sometimes associated with variation in plant height or heading date, with lower Δ commonly associated with reduced stature and later flowering, it was difficult to separate effects of Δ on grain number, yield and biomass independent of plant height and development. For example, reduced plant height (e.g. 4BS and 4DS in C/H and S/T populations) was associated with greater grain number to increase total biomass and grain yield under irrigated conditions (Table 8).

## Discussion

For many genetically complex traits (e.g. biomass, harvest index and grain yield), screening populations under moderate-to severe water limitation (i.e. drought) is difficult owing to: (1) unpredictability in the timing and amount of available water, contributing to genotype × environment interaction (Ceccarelli et al. 1991; Richards et al. 2002); and (2) greater within-site variability contributing to larger sampling variance (Rebetzke et al. 2002). Together these factors decrease genetic variance and reduce heritability to reduce the correlation of phenotype with genotype. Δ is a genetically complex trait for which expression in leaf and other plant tissues varies with water-supply. Low soil water availability lowers stomatal conductance which can reduce genetic variance and heritability for Δ particularly when there is spatial variability in soil water availability (Johnson et al. 1990; Condon et al. 1992; Ehdaie and Waines 1994). Furthermore, reduction in soil water and increasing

**Table 8** Estimated additive (*a*) genetic effect, percent additive genetic variance, and chromosomal location (and corresponding 95% confidence intervals in parenthesis) of QTL for carbon isotope discrimination measured on random DH progenies from the Cranbrook/Halberd, Sunco/Tasman, and CD87/Katepwa populations evaluated across irrigated environments

| Chromosome[a] | Nearest marker | QTL position (cM) | *a* Genetic effect[b] (‰) | Additive genetic variance (%) | Plant height (cm) | Days to heading (days) | Aerial biomass (t ha$^{-1}$) | Grain yield (t ha$^{-1}$) | Grain weight (mg) | Grain number (m$^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Cranbrook/Halberd | | | | | | | | | | |
| 1BS | *gwm666* | 15.3 (9.0–33.6) | −0.05* | 4 | −1.9* | +0.6 | +0.20 | +0.19* | −0.3 | +1,115** |
| 2AS | *wPt-3114* | 43.8 (38.6–50.1) | 0.07** | 9 | +2.8** | −1.8* | +0.30 | −0.03 | +0.9* | −245 |
| 2DS | *ppd1* | 37.7 (20.7–47.7) | 0.12** | 10 | −1.8* | −2.5** | −0.92* | +0.08 | +2.0** | −865* |
| **3BS** | *cdo395* | 54.3 (46.3–63.2) | 0.07* | 5 | +0.4 | −0.4 | −0.26 | −0.16* | −0.8* | +16 |
| **4AS** | *gwm192* | 23.7 (5.0–27.7) | −0.05** | 4 | −2.0* | +0.9* | +0.24 | +0.27** | +0.2 | +706* |
| **4BS** | *gwm495* | 63.4 (59.1–65.6) | −0.08* | 5 | −11.1** | −0.5 | +0.73** | +0.87** | −0.5 | +2,396** |
| **5AS** | *wPt-2768* | 19.0 (9.0–22.7) | 0.05* | 4 | −0.4 | −0.1 | −0.09 | −0.04 | −0.6 | +194 |
| 5AL | *psr426* | 115.3 (111.8–136.4) | −0.04* | 3 | +0.2 | +0.4 | −0.22 | −0.07 | +1.8** | −782** |
| 5BL | *bcd351* | 76.1 (56.5–82.3) | −0.05* | 4 | +0.3 | +1.0* | −0.41* | −0.10 | −0.2 | −271 |
| 6DL | *cmwg684a* | 147.1 (143.0–154.3) | 0.04*** | 2$^{QE}$ | −0.6 | +0.1 | +0.03 | +0.05 | +0.1 | −78 |
| **7AS** | *wmc83* | 66.4 (58.1–77.0) | −0.04*** | 2$^{QE}$ | +2.5* | +0.8*** | +0.46** | +0.02 | −0.9* | +74 |
| **7BS** | *gwm537* | 50.4 (18.8–60.4) | −0.05* | 4 | −0.8* | +0.6 | +0.37* | −0.07 | 0.0 | −159 |
| Mean | | | 20.26 | | 85.1 | 140 | 15.0 | 6.04 | 39.6 | 15,423 |
| Sunco/Tasman | | | | | | | | | | |
| **1BL** | *ksuI27a* | 142.0 (111.9–150.1) | 0.07** | 4 | −1.0 | +0.1 | +0.32* | +0.02 | −0.4 | +134 |
| 1DL | *cdo393* | 70.2 (65.1–72.2) | −0.07** | 5 | −0.8 | +0.7* | −0.50** | −0.41** | +0.4 | −641*** |
| 2AL | *gwm526* | 71.0 (59.1–92.5) | −0.04*** | 3 | −0.4 | +0.3 | −0.40* | −0.20* | −0.3 | −397 |
| **2BS** | *wmc154* | 55.1 (42.3–60.5) | 0.05* | 4$^{QE}$ | −2.4* | +0.1 | −0.15 | −0.12 | −1.8** | +352 |
| 2DL | *bcd266b* | 84.1 (72.8 − 90.3) | 0.06** | 5 | +0.2 | −0.4* | +0.50** | +0.29** | −0.6 | +951** |
| **3BS** | *psr902* | 54.3 (46.3–63.2) | 0.05* | 4$^{QE}$ | +0.2 | +0.1 | +0.52** | +0.10 | −1.1* | +584** |
| **4AS** | *gwm192* | 11.4 (4.0–14.7) | 0.06** | 3$^{QE}$ | +0.8 | −0.6* | −0.17 | +0.08 | 0.0 | +128 |
| **4BS** | *Rht−B1* | 15.6 (7.0–19.4) | −0.06* | 4 | −18.4** | +0.6* | +0.55** | +0.81** | −1.8** | +2894** |
| 4DS | *Rht−D1* | 1.0 (0.0–9.0) | 0.09** | 5 | +21.6** | +0.3 | −0.76** | −0.94** | +0.1 | −2409** |
| 6DL | *scuM06* | 57.8 (46.8–68.5) | 0.07** | 3 | −1.2 | +0.1 | +0.03 | +0.05 | +0.2 | −78 |
| **7AS** | *sun1* | 55.2 (42.8–63.2) | −0.04* | 3$^{QE}$ | +0.1 | −0.1 | −0.04 | −0.16* | +0.3 | −683* |
| **7BS** | *wmc364* | 38.4 (31.3–42.4) | −0.07** | 5 | −0.6 | +0.6* | +0.48* | +0.11 | −0.5 | +532* |
| 7BL | *wmc273* | 128.1 (101.1–146.2) | 0.05* | 4 | +0.8 | +0.2 | −0.07 | +0.04 | +0.1 | −189 |
| Mean | | | 19.10 | | 79.6 | 146 | 14.8 | 6.31 | 38.6 | 16,562 |
| CD87/Katepwa | | | | | | | | | | |
| **1BL** | *psr305* | 177.2 (172.4–181.2) | 0.10** | 8 | −2.7** | −0.5 | −0.13 | +0.02 | +0.5 | −129 |
| **2BS** | *wmc154* | 2.0 (0–14.2) | 0.04*** | 3 | +0.9 | −0.4 | +0.59* | +0.23* | +0.3 | +540* |
| 3BL | *P35/M37−1* | 125.1 (120.0–132.6) | 0.07* | 3 | −2.4** | −0.4 | +0.69** | +0.29** | −0.6 | +1093** |

**Table 8** continued

| Chromosome[a] | Nearest marker | QTL position (cM) | *a* Genetic effect[b] (‰) | Additive genetic variance (%) | Plant height (cm) | Days to heading (days) | Aerial biomass (t ha$^{-1}$) | Grain yield (t ha$^{-1}$) | Grain weight (mg) | Grain number (m$^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| **4AS** | *abg484* | 30.7 (28.7–30.7) | −0.10** | 9 | −3.9** | +0.6* | −0.72** | −0.27** | −0.7* | −372 |
| **4BS** | *ksuC2* | 67.9 (64.3–70.0) | −0.05* | 4 | −16.1** | +0.9* | +0.19 | +0.36** | −2.3** | +2,061** |
| 4DS | *wmc48b* | 12.5 (8.0–20.5) | −0.04* | 3 | +3.9** | +0.7* | −0.21 | −0.10 | +0.3 | −345 |
| **5AS** | *P41/38–5* | 45.7 (30.0–50.3) | 0.05** | 4 | −3.1* | +0.6* | +0.11 | +0.11 | −0.2 | +368 |
| 6BS | *gwm644* | 10.4 (2.0–14.2) | −0.06* | 3 | +0.2 | −1.1* | −0.01 | +0.04 | +1.5** | −437* |
| 7AL | *ksuH9c* | 127.4 (119.2–130.4) | 0.07** | 4 | +0.4 | +1.1* | +0.60* | +0.02 | −1.3* | +475* |
| Mean | | | 19.06 | | 101.1 | 140 | 14.7 | 5.61 | 38.9 | 14,788 |

Additive genetic effects for a range of agronomic traits are also estimated at the nearest linked marker

Positive additive effects indicate that the first parent allele (e.g. Cranbrook, Sunco or CD87) increases the value of the trait

[a] Chromosomes in bold contain Δ QTL which appear to collocate across two or more populations

[b] Additive effect estimated as one-half the difference in homozygotes carrying either parental allele

*, **, *** Indicates marker effect is statistically different from zero at $P = 0.05$, 0.01 and 0.10

QE denotes significant QTL × environment interaction

VPD contributes to differential water use and genotypic rank changes for Δ as the season progresses (Condon et al. 2004). To counter this well-established problem, the current study was conducted mainly under favorable, well-watered conditions. This served to maximize genetic variance and heritability for Δ (Table 2), and improve QTL detection (Beavis 1998). Indeed, heritabilities for Δ were typically high except when evaluated in late-sown (GES 2003 late) or droughted (Gundibindyal 2004) environments (Table 2).

High narrow-sense heritabilities for Δ indicated a strong correlation of phenotype with genotype and the potential for reliable detection of QTL. Evidence for transgressive segregation coupled with gaussian distributions among DH progeny suggested multiple, independent alleles for Δ in all populations. The strong correlation of mid-parent with progeny Δ mean indicated largely additive genetic control repeatable over populations and environments. Similar strong linear associations were observed between mid-parent and progeny Δ means in a separate genetic study encompassing a broad range of parental wheat genotypes varying for Δ (Rebetzke et al. 2006). This predominance of additive gene action is manifest for Δ (Rebetzke et al. 2006) and TE (Malik et al. 1999; Solomon and Labuschagne 2004). Additive gene action reflects the average effect of a gene substitution. The strong evidence for simple additive gene action for Δ indicates that replacement and fixation of desirable alleles within a locus could be readily achieved in selection of lines with altered Δ. It is also conceivable that alleles at loci differing between the three sampled populations could be combined to develop lines in which Δ is altered even further.

Estimation of gene number from the progeny range and variance gave large numbers of segregating genes for Δ in each population. While the robustness of these estimates is contingent on many assumptions (Mather and Jinks 1982), derived estimates were reasonably consistent with the large numbers of significant QTL mapped for Δ in all three populations. These QTL varied in size, accounting for small to modest amounts of the total genotypic variance. Effects were largely additive within a locus with little evidence for any interlocus interaction. A number of Δ chromosomal regions were identified as consistent across two or all three populations. These included QTL on chromosomes 1BL, 3BS, 4AS, 4BS, 4DS, 5AS and 7AS. QTL common to populations may have greater potential use for marker-assisted breeding. QTL were also identified unique to specific populations. This was not unexpected given the small pedigree-relatedness among the three populations ($\theta_{AB} = 0.08$–$0.26$; Kammholz et al. 2001).

There are no other reports in the literature of QTL for Δ in wheat. QTL have been reported for Δ across a range of plant species including barley (*H. vulgare*) (Teulat et al.

2002), *Arabidopsis thaliana* (Hausmann et al. 2005; Masle et al. 2005), rice (*O. indica*) (Price et al. 2002; Laza et al. 2006), and cotton (*G. hirsutum*) (Saranga et al. 2001). Genetic control of Δ in all four species was commonly associated with variation at many independent loci (e.g. up to 9–11 in rice and cotton, respectively), each accounting for small genic variation and individually accounting for less than 15% of the phenotypic variance. Where reported, most QTL were not repeatable across years and so were subject to large QTL × environment interaction (e.g. Saranga et al. 2001; Price et al. 2002). Further, QTL have commonly been reported for single populations only. One exception was Hausmann et al. (2005), where only one of the seven detected Δ QTL was repeatable across two populations derived from the common parent, Landsberg *erecta*. The present study on wheat has identified QTL common across environments and unrelated populations.

The large number of wheat Δ QTL reported herein is consistent with Rebetzke et al. (2006), where multiple alleles were identified segregating for Δ in a large wheat diallel. Similarly, the low frequency of reduced Δ progeny in biparental populations derived from the low Δ donor, cv. Quarrion, is consistent with the accumulation of many genes of small effect (Rebetzke et al. 2002, 2006). Some of this variation may reflect segregation at loci associated with leaf conductance and/or photosynthetic capacity, both of which affect Δ and are known to be under polygenic control (Simón 1994; Hervé et al. 2001; Rebetzke et al. 2003). In addition, many genes, both major and minor in effect, are distributed across the wheat genome to affect plant height and development. The *Rht-B1b* and *Rht-D1b* dwarfing genes have been shown to reduce Δ in wheats of spring (Richards 1992) and winter (Morgan et al. 1990) habit. Similarly, days to heading and Δ were strongly, negatively correlated ($r = -0.62$ to $-0.97$) for spring wheats grown under droughted and well-watered conditions (Ehdaie et al. 1991). Genotypic variation in Δ was closely linked to flowering time ($r_g = 0.98$) in *Arabidopsis* (McKay et al. 2003), common bean and cowpea (Hall et al. 1994), while most of the Δ QTL previously reported for a barley mapping population co-segregated with QTL for heading date and/or plant height (Forster et al. 2004). Given that Δ in our study was obtained from leaves sampled at Z31 (i.e. c. 6–8 weeks prior to heading), and given the relatively small range in heading date in all three populations (c. 5–10 days), the physiological basis for the relationship between Δ and plant height and flowering is unclear. Cell and leaf size is reduced in dwarf wheat isolines to increase specific leaf weight, chlorophyll content and photosynthetic rate, in turn reducing leaf Δ (Morgan et al. 1990). Reductions in cell size have also been associated with delayed heading date in winter wheats (Limin and Fowler 2001). Thus it is conceivable that the smaller cell size commonly associated with developmental and height-reducing genes increases chlorophyll content and leaf nitrogen per unit area to increase photosynthetic capacity, an important component of transpiration efficiency (Condon et al. 2004).

Variation in development and plant height has been shown to affect Δ across different plant species (Ehdaie et al. 1991; Hall et al. 1994; McKay et al. 2003; Forster et al. 2004; Laza et al. 2006; Takai et al. 2006). Phenology and stature also affect plant growth to change biomass, harvest index and water use under drought, particularly when drought is terminal (Richards et al. 2002). Where variation in development and stature exist and contribute to some of the genotypic differences in Δ, they are likely to confound and compromise the influence of genotypic differences in TE on grain yield and biomass under drought. This will also confound interpretation of effects of TE on yield when the Δ signal is measured in grain and the pre- and post-anthesis assimilation contribution to grain Δ cannot be separated. The potential for variation in development and height to affect TE in wheat is substantial given the many major and minor developmental and height genes that may segregate in a wheat cross (e.g. Snape et al. 2001). That said, in the $BC_2$-derived low and high Δ contrast reported in Rebetzke et al. (2002), heading date and plant height were constrained to be similar for low and high Δ sister lines. This allowed the effects of Δ on yield and biomass to be assessed independently of those other major factors predisposing adaptation.

Removal of height and development effects through covariance analysis demonstrated that genotypic increases in Δ were associated with small to moderate changes in biomass and harvest index, and grain yield. Increased grain number was the major yield component contributing to this increase. Similar associations between grain yield and grain number have been observed for wheat and barley genotypes grown under favorable and water-limited conditions (e.g. Ehdaie et al. 1991; Morgan et al. 1993; Araus et al. 2003; Condon et al. 2004; Rebetzke et al. 2002). Fischer et al. (1998) reported a strong, positive relationship ($r = 0.71$) for Δ and yield of CIMMYT wheat varieties representing 30 years of breeding in irrigated environments. Again, most of this 30-year yield progress at CIMMYT was associated with increased grain number. This highlights the potential for selection of increased Δ in well-watered environments to improve grain yield (Condon et al. 1987, 2007).

This study has confirmed the polygenic inheritance of Δ in wheat through the identification of a number of QTL for Δ. These QTL were often repeatable across environments and in some cases across populations. Genetic effects were typically small, reducing the value of these QTL in marker-assisted selection. Thus it may be difficult to justify the use of

MAS for independent QTL of small effect when genetic gain through phenotypic selection is high. Further, the different QTL associated with variation for Δ across populations may make the identification of robust markers difficult across different gene pools. However, high narrow-sense heritabilities for Δ observed in all three populations increases confidence in direct selection among progeny for altered Δ.

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