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Influence of the Gibberellin-sensitive *Rht8* Dwarfing Gene on Leaf Epidermal Cell Dimensions and Early Vigour in Wheat (*Triticum aestivum* L.)

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• *Background and Aims* The gibberellin-insensitive *Rht-B1b* and *Rht-D1b* dwarfing genes are known to reduce the size of cells in culms, leaves and coleoptiles of wheat. Resulting leaf area development of gibberellin-insensitive wheats is poor compared to standard height (*Rht-B1a* and *Rht-D1a*) genotypes. Alternative dwarfing genes to *Rht-B1b* and *Rht-D1b* are available that reduce plant height, such as the gibberellin-responsive *Rht8* gene. This study aims to investigate if *Rht8* has a similar dwarfing effect on the size of leaf cells to reduce leaf area.
• *Methods* The effect of *Rht8* on cell size and leaf area was assessed in four types of epidermal cells (interstomatal, long, sister and bulliform) measured on leaf 2 of standard height (*rht8*) and semi-dwarf (*Rht8*) doubled-haploid lines (DHLs). The DHLs were derived from a cross between very vigorous, standard height (*rht8*) ('Vigour18') and less vigorous, semi-dwarf (*Rht8*) ('Chuan-Mai 18′) parents.
• *Key Results* Large differences were observed in seedling vigour between the parents, where 'Vigour18' had a much greater plant leaf area than 'Chuan-Mai 18'. Accordingly, 'Vigour18' had on average longer, wider and more epidermal cells and cell files than 'Chuan-Mai 18'. Although there was correspondingly large genotypic variation among DHLs for these traits, the contrast between semi-dwarf *Rht*8 and tall *rht*8 DHLs revealed no difference in the size of leaf 2 or average cell characteristics. Hence, these traits were independent of plant height and therefore *Rht8* in the DHLs. Correlations for leaf and average cell size across DHLs revealed a strong and positive relationship between leaf width and cell files, while the relationships between leaf and cell width, and leaf and cell length were not statistically different. The relative contribution of the four cell types (long, sister, interstomatal and bulliform) to leaf size in the parents, comparative controls and DHLs is discussed.
• *Conclusions* Despite a large range in early vigour among the DHLs, none of the DHLs attained the leaf area or epidermal cell size and numbers of the vigorous *rht8* parent. Nonetheless, the potential exists to increase the early vigour of semi-dwarf wheats by using GA-sensitive dwarfing genes such as *Rht8*.

Key words: Wheat, *Triticum aestivum* L., gibberellic acid, leaf epidermal cells, doubled-haploids, early vigour, leaf area, alternative dwarfing genes.

INTRODUCTION

Improved seedling establishment and rapid leaf area development contribute to greater ground cover early in the season. In water-limited environments, such as in Mediterranean climates, poor ground cover will reduce competitiveness with weeds and increase water loss through soil evaporation. In turn, water use efficiency, biomass and ultimately grain yield are likely to decrease (Richards and Townley-Smith, 1987; Botwright *et al*., 2002). In barley and triticale, a big embryo and high specific leaf area contribute to a greater number of large leaf epidermal cells to increase leaf width and therefore leaf area (López-Castañeda *et al*., 1996). Leaf width is an important component of leaf area growth, which, because of its high degree of genetic determination, can be used as a selection criterion in breeding for greater early vigour in wheat (Rebetzke and Richards, 1999; Rebetzke *et al*., 2004).

The *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) gibberellin-insensitive dwarfing genes are widely used to reduce plant height and increase grain yield in wheat breeding programs. These genes confer insensitivity to endogenous gibberellins to decrease cell wall extensibility (Keyes *et al*., 1990), and reduce epidermal cell length compared to standard height (*rht*) genotypes (Keyes *et al*., 1989; Hoogendoorn *et al*., 1990). This reduction in cell length has been established in one cell type, yet several classes of epidermal cells are known to contribute to leaf area development. Furthermore, epidermal cell size and number are known to vary spatially (Beemster and Masle, 1996; Wenzel *et al*., 1997*a*).

The smaller cell sizes associated with *Rht-B1b* and *Rht-D1b* produce concomitant reductions in sub-crown internode and coleoptile length, and leaf area of wheat seedlings (Allan *et al*., 1961; Allan, 1989; Botwright *et al*., 2001). A number of alternative dwarfing genes (*Rht4* to *Rht20*) have been reported to reduce plant height in wheat but show sensitivity to exogenous gibberellic acid (GA) (Gale and Youssefian, 1985; Ellis *et al*., 2004). Unlike *Rht-B1b* and *Rht-D1b*, many of the GA-sensitive, height-reducing genes, such as *Rht8*, do not shorten coleoptile length or decrease seedling vigour (Rebetzke *et al*., 1999; Botwright *et al*., 2001; Ellis *et al*., 2004). There is no published information on the influence of the GA-sensitive dwarfing genes on epidermal cell size and associated effects on variation in leaf area of wheat. This paper reports on two experiments investigating the influence of the

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TABLE 1. *Plant height at maturity and dwarfing genes of parents, comparative controls and DHLs used in experiments 1 and 2*

Semi-dwarf					Tall				
Genotype	Dwarfing gene	GA	Experiment	Height (cm)	Genotype	Dwarfing gene	GA	Experiment	Height (cm)
Parents									
'Chuan-Mai 18'	*Rht8*	+	1	60	'Vigour18'	*rht*8	+	1	120
Comparative controls									
'Amery'	*Rht-D1b*	–	1	60	'Halberd'	*Rht-D1a*	+	1	90
'Stiletto'	*Rht-D1b*	–	1	65					
'Westonia'	*Rht-D1b*	–	1	60					
DHLs									
B23	*Rht8*	+	2	68	A5	*rht8*	+	2	96
B31	*Rht8*	+	1,2	69	A11	*rht8*	+	1,2	125
C5	*Rht8*	+	1,2	65	A35	*rht8*	+	1,2	99
C7	*Rht8*	+	2	59	A37	*rht8*	+	2	120
C18	*Rht8*	+	2	70	B13	*rht8*	+	2	97
C32	*Rht8*	+	1,2	67	B21	*rht8*	+	1,2	96
DHL mean				66					106

Plant height of semi-dwarf and tall DHLs were significantly different at $P = 0{\cdot}01$ using a *t*-test for two-sample means. Gibberellic acid (GA)-sensitivity is designated as (+) sensitive and (–) insensitive.

gibberellin-sensitive *Rht8* dwarfing gene on the size and number of four leaf epidermal cell types, and their relationship with leaf area in seedlings of related wheat genotypes.

MATERIALS AND METHODS

Wheat lines and growth conditions

Early leaf area development and the number and size of leaf epidermal cells were examined in gibberellin (GA)-sensitive parents and semi-dwarf (*Rht*8) or tall (*rht*8) doubled-haploid lines (DHLs), and compared to tall, GA-sensitive and semi-dwarf, GA-insensitive comparative controls. Parental lines included the vigorous, tall, 'Vigour18' (*rht8*), and the less vigorous, semi-dwarf 'Chuan-Mai 18' (*Rht8*) wheats. 'Vigour18' has been bred and selected as a source of extremely high seedling vigour (Richards and Lucaks, 2002). All DHLs were randomly chosen, except for presence of the *Rht8* dwarfing gene, from a population containing 190 individual lines. Comparative controls included the tall, GA-sensitive variety 'Halberd' (*Rht-D1a*) and semi-dwarf, GA-insensitive varieties 'Amery', 'Stiletto' and 'Westonia' (all *Rht-D1b*). Plant height at maturity for the parents, comparative controls and DHLs are shown in Table 1.

For both experiments, seed were sized to between 45–50 mg before length and breadth of the embryo was measured using a Leica® stereomicroscope. Embryo size was subsequently estimated as embryo length × breadth (Moore *et al*., 2001). Seed were then sown at a depth of 20 mm into trays (600 × 300 × 120 mm) containing a fertile potting mix (2 : 1 : 1 sand : peat : vermiculite). Plants were grown in a growth cabinet with a day/night regime of 15/10 °C, 70/80 % relative humidity and a 10/14 h day/night length, with a photon flux density of 350 μmol m^2 s^{-1}.

Seed and plant analyses

Plants were harvested at the 3·5 leaf stage (at 28 d after sowing), and divided into mainstem and tillers, leaves, and stems. The length and width of mainstem leaves was measured with a ruler and total leaf area calculated after Rebetzke and Richards (1999). All plant parts, with the exception of leaf 2, which was used for measurements of cell dimensions, were dried at 60 °C for 3 d before weighing.

Leaf 2 was prepared for microscopy by clearing the leaf of chlorophyll by immersion in approximately 20 mL of methanol in a capped 25 mL glass vial for 12 h at 4 °C. The methanol was then substituted with lactic acid for indefinite storage at room temperature. The clearing procedure partly destroys cytoplasm and removes chlorophyll without damaging cell walls (Beemster and Masle, 1996). Cleared leaf-2 blades were cut transversely into three segments (distal, medial and basal) of approximately equal length for measurements of cell size in whole mounts.

The epidermal cells between two veins consist of two rows of interstomatal cells (IS) containing the guard and adjacent sister cells (S) that are derived from the same mother cells as the subsidiary cells. Unspecialized, elongated cells (L) lie between the two innermost rows of sister cells on the abaxial surface, and bulliform cells (B) on the adaxial surface. For each leaf segment (distal, medial and basal), the length and width of ten adjacent cells ('cell within segment') of each of four epidermal cell types (elongated, interstomatal, sister and bulliform cells), located between the first and second vein from the midrib, were measured using an eyepiece graticule mounted in a Zeiss® microscope and set at 200× magnification. The long cells exceeded the field of view at high magnification and were instead measured at 100×. The locations of each cell type on the abaxial or adaxial leaf epidermis are shown in Fig. 1. The number of cell files across the leaf was counted only in the medial leaf segment and the number of cells in each cell file calculated as (leaf segment length)/(cell length).

Experimental design and statistical analyses

Two experiments were undertaken. In experiment 1, genotypes included the parents, 'Vigour18' (*rht*8) and

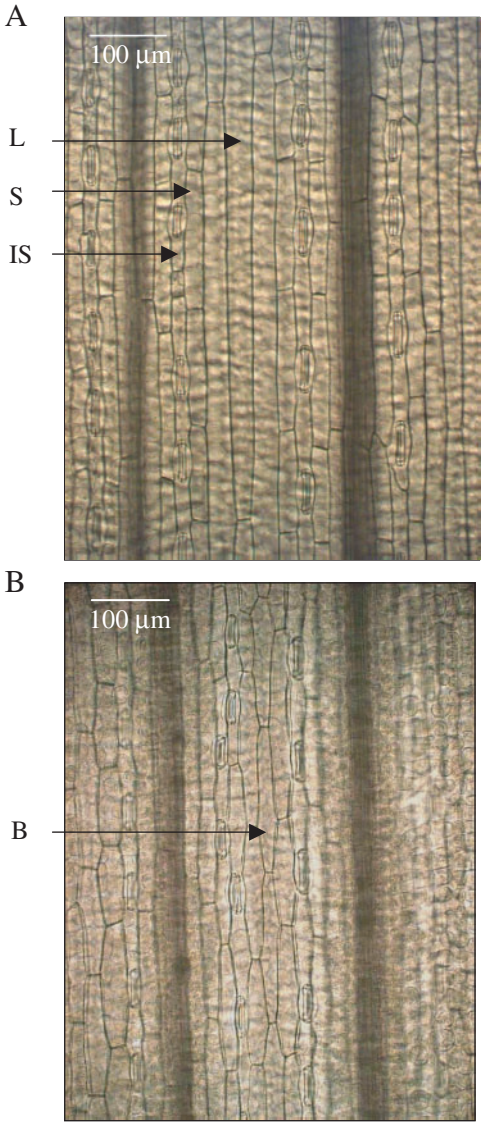

FIG. 1. (A) Abaxial and (B) adaxial leaf surfaces of wheat, detailing the four measured cell types. Abbreviations: L, long; IS, interstomatal; S, sister; B, bulliform cells. Magnification 200×.

'Chuan-Mai 18' (*Rht*8), three semi-dwarf (*Rht*8) and three tall (*rht*8) DHLs, and the four comparative controls, 'Halberd', 'Amery', 'Stiletto' and 'Westonia' (Table 1). Plants were grown in a randomised complete block design (RCBD) with four replicates. Data for seedling vigour and average cell characteristics were analysed using the generalised linear models procedure GLM in SAS (SAS, 1990). Pre-planned treatment contrasts were constructed to test for statistical differences between semi-dwarf (*Rht8*) and tall (*rht8*) DHLs; the parents, 'Chuan-Mai 18' (*Rht8*) and 'Vigour18' (*rht8*); and between GA-sensitive ('Halberd', 'Vigour18', 'Chuan-Mai 18') and GA-insensitive ('Amery', 'Stiletto', 'Westonia') wheat genotypes. Data for genotype, leaf segment, cell within segment and their interaction for length and width of the four cell types (long, sister, interstomatal and bulliform cells) were analysed with cell nested within leaf segment.

In experiment 2, the number of *Rht8 and rht8* DHLs was expanded to include an additional three DHLs per height group (i.e. six of each in total) (Table 1) to more adequately assess the nature and extent of relationships between the different cell components and leaf area. Experimental design, methodology and statistical analyses were the same as for experiment 1, except the parents and control genotypes were omitted. The significance of correlations between length and width of the four cell types, average cell length and width, cell files and leaf length and width for the 12 DHLs was analysed using the Pearson moment correlation procedure CORR in SAS (SAS, 1990).

RESULTS

Experiment 1

Leaf cellular characteristics, averaged across all cell types, are presented for all genotype groups in Table 2, while the length, width and, where calculable, number of the four separate cell types are presented in Table 3. In general, the long cells, which were situated between two parallel veins on the lower surface of the leaf (Fig. 1), were on average approx. 4-fold longer and around 25 % wider than the sister and interstomatal cells, respectively (Table 3). The bulliform cells, found on the upper leaf surface and responsible for leaf rolling in monocotyledons, were similar in length to the sister cells, but, at around 50 µm in width, were the widest of the four cell types (Table 3).

Effects of leaf segment and cell within segment on the length and width of the four cell types are shown in Table 3 and Fig. 2 (A, B). Long and sister cells were shorter in distal, compared to basal segments (Fig. 2A). In contrast, bulliform cells were longer in medial compared to basal and distal leaf segments (Fig. 2A). The widths of all cell types were widest in basal leaf segments (Fig. 2B). Positional effects of the ten cells measured in each leaf segment ('cell within segment' in Table 3) were significant only for sister cell length (Table 3). There were no interactions between genotype or treatment contrasts (*Rht8 vs. rht8*; parents; or GA+ *vs.* GA–) with leaf segment or cell within segment.

Parental lines (experiment 1)

'Vigour18' produced exceptionally long and wide leaves, which together contributed to a 71 % increase in leaf 2 area, compared to 'Chuan-Mai 18' (Table 2). 'Vigour18' had, on average, longer (35 %), wider (27 %) and, in particular, more cell files across and along (both 25 %) the leaf epidermis than 'Chuan-Mai 18' (Table 2). Of the four cell types measured, only long-cell length was significantly greater (Table 3). There were considerably more sister (24 %), long (20 %) and bulliform (32 %) cells per cell file in 'Vigour18' than 'Chuan-Mai 18' (Table 3).

Comparative controls (experiment 1)

One *Rht-D1a* (tall, GA-sensitive) and three *Rht-D1b* (semi-dwarf, GA-insensitive) genotypes were included as

TABLE 2. *Experiment 1. Seedling vigour and leaf cell characteristics for three semi-dwarf* (Rht8) *and three standard height* (rht8) *doubled-haploid lines, their parents, 'Chuan-Mai 18'* (Rht8) *and 'Vigour18'* (rht8), *and three* Rht-D1b (*'Amery', 'Stiletto' and 'Westonia'*) *and* Rht-D1a (*'Halberd'*) *controls*

		Seedling vigour				Average cell characteristics			
Rht groups	GA	Leaf 2 length (mm)	Leaf 2 width (mm)	Leaf 2 area (cm^2)	Total leaf area (cm^2)	Cell length (μm)	Number of cells/leaf length	Cell width (μm)	Cell files/ leaf width
Doubled-haploids									
Rht8	+	172	5·8	8·0	43·1	393	435	37	173
rht8	+	175	6·0	8·4	35·5	374	466	38	175
Parents									
'Chuan-Mai 18' (*Rht8*)	+	159	6·0	7·6	41·6	350	458	38	173
'Vigour18' (*rht8*)	+	215	7·6	13·0	56·2	374	571	39	216
Comparative controls									
Rht-D1a	+	164	6·3	8·2	46·6	428	403	38	182
Rht-D1b	−	170	5·8	7·9	39·8	373	448	38	188
Contrasts									
Rht8 vs. rht8		n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Parents		***	*	**	n.s.	***	***	**	***
GA+ *vs.* GA−		n.s.	**	*	*	n.s.	n.s.	n.s.	n.s.

Gibberellic acid (GA)-sensitivity is designated as (+) sensitive and (−) insensitive. Treatment contrasts were constructed to test the significance of dwarfing gene effects (*Rht*) in the doubled-haploid lines, parents and GA-sensitivity in the parents and comparative controls.
* $P = 0{\cdot}05$, ** $P = 0{\cdot}01$, *** $P = 0{\cdot}001$; n.s., not statistically significant at $P = 0{\cdot}05$.

TABLE 3. *Experiment 1. Leaf cellular dimensions of four cell types (interstomatal, sister, long and bulliform cells) for three semi-dwarf* (Rht8) *and three tall* (rht8) *doubled-haploid lines, their parents, 'Chuan-Mai 18'* (Rht8) *and 'Vigour18'* (rht8), *three* Rht-D1b *('Amery', 'Stiletto' and 'Westonia') and one* Rht-D1a *('Halberd') comparative controls*

		Interstomatal		Sister			Long			Bulliform		
Rht groups	GA	Length (μm)	Width (μm)	Number/leaf length	Length (μm)	Width (μm)	Number/leaf length	Length (μm)	Width (μm)	Number/leaf length	Length (μm)	Width (μm)
Doubled-haploids												
Rht8	+	166	33	588	299	35	202	870	42	715	246	47
rht8	+	147	33	614	287	37	203	865	44	833	207	50
Parents												
'Chuan-Mai 18' (*Rht8*)	+	156	33	621	256	36	214	745	44	694	229	50
'Vigour18' (*rht8*)	+	145	32	770	281	39	256	845	46	915	236	51
Comparative controls												
Rht-D1a	+	178	33	495	346	37	194	880	45	606	281	52
Rht-D1b	−	156	32	550	307	38	214	825	45	748	230	50
Position												
Segment		n.s.	***	–	***	***	–	***	***	–	***	***
Cell within segment		n.s.	n.s.	–	*	n.s.	–	n.s.	n.s.	–	n.s.	n.s.
Contrasts												
Rht8 vs. rht8		*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	***	*
Parents		n.s.	n.s.	***	n.s.	n.s.	*	**	n.s.	***	n.s.	n.s.
GA+ *vs.* GA−		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.

Gibberellic acid (GA)-sensitivity is designated as (+) sensitive and (−) insensitive. Cell number was derived from leaf length/cell length, exclusive of interstomatal cells. The significance of position on cell size for leaf segment (basal, distal and medial) and cell within segment is shown. Treatment contrasts were constructed to test the significance of dwarfing gene effects (*Rht*) in the doubled haploid lines, parents and GA-sensitivity in the parents and controls.
* $P = 0{\cdot}05$, ** $P = 0{\cdot}01$, *** $P = 0{\cdot}001$; n.s., not statistically significant at $P = 0{\cdot}05$.

comparative controls in experiment 1. Data was analysed together with the parents, to provide orthogonal contrasts of three GA-insensitive ('Amery', 'Stiletto' and 'Westonia') with three GA-sensitive ('Chuan-Mai 18', 'Halberd' and 'Vigour18') genotypes. Leaf 2 width and area, and total leaf area were larger in GA-sensitive genotypes than GA-insensitive genotypes (Table 2). The greater leaf length and area for leaf 2 of 'Vigour18' contributed to the greater vigour of the GA-sensitive genotypes (Table 2). Average cell characteristics of both groups were the same (Table 2). Of the four cell types, only bulliform cell length was greater in GA-sensitive compared to GA-insensitive controls, while the length, width and number of cells were otherwise the same (Table 3).

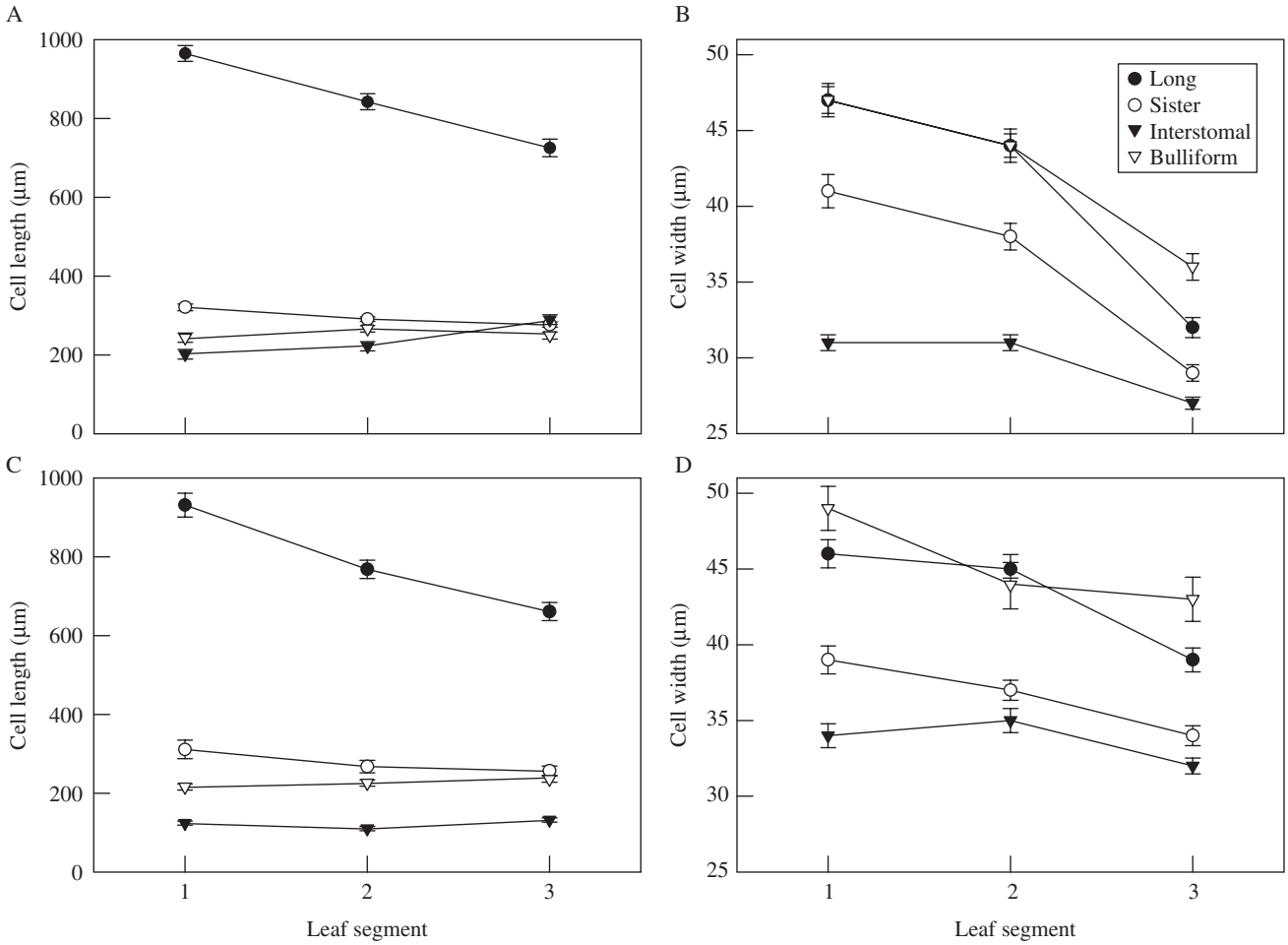

FIG. 2. Effect of leaf segment on average cell length for different cell types: (A, B) experiment 1; (C, D) experiment 2. Leaf segments are: (1) Basal; (2) Medial; and (3) Distal. Bars represent the standard error for the mean.

DHLs (experiment 1)

The size of leaf 2, total leaf area and average cell characteristics of the *Rht8 and rht8* DHLs were similar, with the exception of fewer cells per cell file in the semi-dwarf *Rht8* compared to tall *rht8* DHLs (Table 2). Of the four cell types, interstomatal and bulliform cells were longer, in semi-dwarf *Rht8* compared to tall *rht8* DHLs, yet the bulliform cells were narrower and fewer in number (Table 3). Comparing the DHLs with their parents, average cell length of the DHLs was similar to 'Vigour18', while average cell number along the length of the leaf and number of files across the leaf were similar to 'Chuan-Mai 18' (Table 2).

DHLs (experiment 2)

An additional six DHLs were used, together with the original DHLs, in a second experiment to evaluate the nature and extent of relationships between the components of leaf size and the number and dimensions of the different leaf epidermal cell types. There were significant genotypic differences in leaf length and width among all 12 DHLs (Table 4). Specifically, long cells varied in all dimensions across genotypes, with sister, interstomatal and bulliform cells showing variation in either cell number, length or width (Table 5).

The contrast between semi-dwarf *Rht8* versus tall *rht8* DHLs revealed no difference in the average length and width of leaf 2 or average cell characteristics, although semi-dwarf *Rht8* DHLs produced a larger total leaf area (Table 4). Nonetheless, there were differences in the width and length of interstomatal cells, which were 11 % and 3 % longer and wider, respectively, in semi-dwarf *Rht8* than tall *rht8* DHLs (Table 5). Cell dimensions and numbers of long, interstomatal and bulliform cells were otherwise the same across the two genotype groups (Table 5).

Effects of leaf segment and cell within segment on the length and width of the four cell types is shown in Table 5 and Fig. 2 (C, D). The length and width of long and sister cells were shorter, in distal compared to basal leaf segments, as in experiment 1 (Fig. 2C). The length of interstomatal and bulliform cells were more uniform across the three leaf segments (Fig. 2C). Interactions between genotype, contrast and leaf segment were not significant ($P > 0{\cdot}05$) for length and width of the four cell types.

TABLE 4. *Experiment 2. Average seedling vigour and cell characteristics for leaf 2 for six semi-dwarf* (Rht8) *and six tall* (rht8) *doubled-haploid wheats*

	Seedling vigour				Average cell characteristics			
Rht groups	Leaf 2 length (mm)	Leaf 2 width (mm)	Leaf 2 area (cm^2)	Total leaf area (cm^2)	Cell length (μm)	Number cells/leaf length	Cell width (μm)	Cell files/leaf width
Doubled-haploids								
Rht8	145	6·3	7·3	34·4	342	426	35	179
rht8	142	6·3	7·3	27·1	339	423	34	179
Genotype	**	***	***	***	***	***	n.s.	*
Contrasts								
Rht8 vs. rht8	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.

The significance of genotype, across all DHLs, is shown. Treatment contrasts were constructed to test the effect of dwarfing genes (*Rht*) in the doubled haploid lines.
* $P = 0{\cdot}05$, ** $P = 0{\cdot}01$, *** $P = 0{\cdot}001$; n.s., not statistically significant at $P = 0{\cdot}05$.

TABLE 5. *Experiment 2. Leaf cellular dimensions of four cell types (interstomatal, sister, long and bulliform cells) for six semi-dwarf* (Rht8) *and six tall* (rht8) *doubled-haploid wheats*

	Interstomatal		Sister			Long			Bulliform		
Rht groups	Length (μm)	Width (μm)	Number/leaf length	Length (μm)	Width (μm)	Number/leaf length	Length (μm)	Width (μm)	Number/leaf length	Length (μm)	Width (μm)
Doubled-haploids											
Rht8	128	30	662	224	34	191	782	41	642	233	42
rht8	115	29	629	227	33	182	791	41	649	221	43
Genotype	**	n.s.	*	n.s.	n.s.	***	***	**	n.s.	n.s.	***
Position											
Segment	*	***	–	n.s.	***	–	***	***	–	*	***
Cell within segment	**	n.s.	–	n.s.	n.s.	–	***	***	–	n.s.	n.s.
Contrast											
Rht8 vs. rht8	***	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

The significance of genotype, leaf segment and cell within segment are shown. Treatment contrasts were constructed to test the effect of dwarfing genes (Rht8 *vs.* rht8) in the doubled-haploid lines.
* $P = 0{\cdot}05$, ** $P = 0{\cdot}01$, *** $P = 0{\cdot}001$; n.s., not statistically significant at $P = 0{\cdot}05$.

Pearson moment correlations between length and width of the four cell types, average cell dimensions and the length and width of leaf two were computed across the 12 DHLs (Table 6). Leaf width was strongly and positively correlated with average cell files across DHLs (Table 6, Fig. 3). Similarly, leaf length was positively correlated with average cell number per file, although this relationship may be somewhat biased as the average cell number per file was derived from the leaf length divided by the average cell length. There was no correlation between leaf length and average cell length or between leaf width and average cell width (Table 6), even though the length and width of leaf 2 and cell length showed significant variation across the DHLs (Table 4).

The length and width of the four cell types were not correlated with the length and width of leaf 2 (Table 6). Of the four cell types, only the long and sister cells contributed significantly to variation in average cell length and average cell width (Table 6). The width and length of both these cell types were strongly and positively correlated with average cell length and width and negatively correlated with average cell number (Table 6). The only significant relationships observed among the dimensions of the four individual cell types were strong positive correlations between sister and interstomatal cell widths, and sister and long cell lengths (Table 6).

DISCUSSION

The use of alternative dwarfing genes, such as *Rht8*, has been proposed as a means of maintaining semi-dwarf stature and high yield potential in wheat, whilst allowing selection for greater early vigour and coleoptile length (Rebetzke and Richards, 1999, 2000; Botwright *et al*., 2002; Ellis *et al*., 2004). In this study, we observed large differences in seedling vigour between the parents. The tall (*rht8*) 'Vigour18', which had been specifically bred for high vigour (Richards and Lucaks, 2002) had a much greater leaf 2 size and area per plant than semi-dwarf (*Rht8*) 'Chuan-Mai 18'. Accordingly, 'Vigour18' had on average longer, wider and more epidermal cell numbers and files than 'Chuan-Mai 18'. Similarly, there was large variation across the DHLs for

TABLE 6. *Experiment 2. Correlations* (r-*values*) *among leaf cellular dimension and leaf 2 size descriptors for 12 doubled-haploids* (Rht8 *and* rht8)

	Width of cell type			Length of cell type				Average of cell type				Leaf 2	
	S	IS	B	L	S	IS	B	Number	Length	Width	Files	Width	Length
Width of cell type													
L	0·37	−0·07	0·11	−0·40	0·01	−0·04	−0·26	0·56	−0·37	0·80**	0·28	0·11	0·53
S		0·73**	−0·10	0·11	0·32	0·11	0·35	0·14	0·18	0·82**	−0·16	0·01	0·49
IS			−0·06	0·29	0·12	0·22	0·49	0·02	0·35	0·50	−0·01	0·27	0·45
B				0·04	0·06	−0·26	−0·16	0·26	−0·01	−0·04	0·42	0·49	0·47
Length of cell type													
L					0·73**	0·28	0·25	*−0·79***	0·98***	−0·22	−0·25	−0·09	−0·11
S						0·24	0·05	*−0·61**	0·76**	0·09	−0·31	−0·31	−0·01
IS							0·19	−0·45	0·41	0·06	−0·30	−0·43	−0·24
B								−0·32	0·37	0·07	−0·16	0·29	−0·16
Average of cell type													
Number									*−0·82***	0·49	0·44	0·42	*0·67**
Length										−0·15	−0·30	−0·13	−0·14
Width											0·12	0·14	0·66*
Files												0·71**	0·35
Leaf 2													
Width													0·48

Abbreviations: L, long cells; S, sister cells; IS, interstomatal cells; B, bulliform cells. Average cell number was calculated from leaf length/cell length and is biased and data is shown in italics.
* $P = 0{\cdot}05$, ** $P = 0{\cdot}01$, *** $P = 0{\cdot}001$.

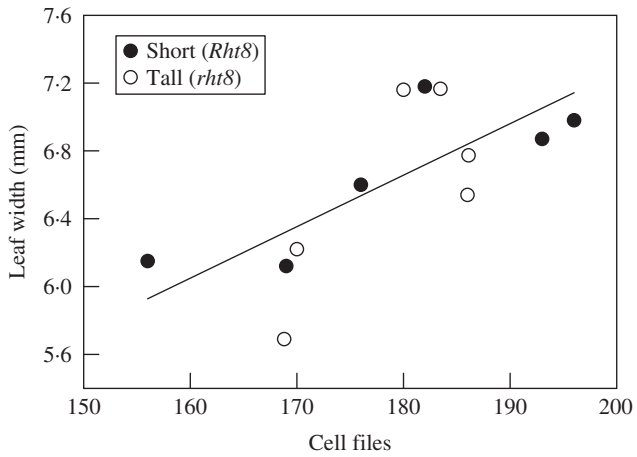

FIG. 3. Effect of leaf cellular dimensions on leaf width (LW) versus cell files (CF) of short (*Rht8*) and tall (*rht8*) doubled-haploids; LW = 1·18 + 0·030CF, $r = 0{\cdot}71$, $P = 0{\cdot}01$.

these traits. Yet in the contrast between semi-dwarf *Rht8* and tall *rht8* DHLs, leaf 2 size and the average cell characteristics did not differ and were therefore independent of plant height. The implications of these findings and the relative contribution of the four cell types of long, sister, interstomatal and bulliform cells to leaf size in the parents, comparative controls and DHLs warrant further discussion.

The contribution of the four types of epidermal cells and leaf segment to seedling vigour

The leaf epidermis consists of more than one cell type, the sizes of which vary considerably along the length and across the breadth of the blade (Beemster and Masle, 1996; Wenzel *et al*., 1997*a*). Characterizing leaf length using only one type of cell (Keyes *et al*., 1989; Hoogendoorn *et al*., 1990) is not representative of the complexity of the leaf epidermis, where length, width and number of cells varies both spatially and genotypically, as indicated in barley (Wenzel *et al*., 1997*b*). Here, the four types of leaf epidermal cells each contributed differently to leaf size for each genotype. For example, longer leaves in the *rht8* parent, 'Vigour18', were associated with longer long-cell types, yet bulliform and sister cells were approximately the same length but more numerous. Consequently, increased lengths of long and sister cells were compensated by a reduction in cell number so that there was no overall effect on leaf length.

Epidermal cell length and width varied along the leaf blade. Wenzel *et al*., (1997*b*) have reported similar reductions in cell length along the leaf blade in barley. In contrast, cell length of 'elongated cells' (equivalent here to long cells) of wheat measured by Beemster and Masle (1996) under different soil strengths were relatively similar along the leaf blade, while bulliform cells showed a similar response to the present study. Regardless, there were no interactions between genotype or *Rht* group (i.e. contrast) and leaf segment for the different cell types.

Parental lines

Early vigour of the *rht8* parent, 'Vigour18', was exceptional compared to the mean of its DH progeny, the *Rht8* parent 'Chuan-Mai 18' and the comparative controls. This vigour was largely associated with a greater number of cell files across the leaf and more cells along the leaf and, to a lesser extent, with wider and longer cells. The contribution of cell file number to the leaf area of 'Vigour18' is similar to

barley, where wider leaves are associated with larger embryos and more cell files, whereas the wider leaves of other cereals, such as oats, is due, in part, to wider cells (López-Castañeda *et al*., 1996). In comparison, the GA-insensitive dwarfing genes increase cell width in the leaf sheaths of wheat but not the leaf blade (Keyes *et al*., 1989).

Comparative controls

It has been argued that selection for greater seedling vigour may be constrained if GA-insensitive dwarfing genes are maintained (Rebetzke and Richards, 2000). The GA-insensitive *Rht-B1b* and *Rht-D1b* semi-dwarf wheats show reduced cell wall extensibility (Keyes *et al*., 1990). In turn, the length of the leaf extension zone is decreased to produce shorter cells (Keyes *et al*., 1989; Hoogendoorn *et al*., 1990; Tonkinson *et al*., 1995) and slower rates of leaf elongation (Ellis *et al*., 2004). It is also reported that *Rht-B1b* and *Rht-D1b* genotypes have fewer cell files (López-Castañeda *et al*., 1996) and hence narrower leaves.

In the current study, leaves of *Rht-D1b* comparative controls were similar in size to both DHLs and the *Rht8* parent, although smaller than the vigorous *rht8* parent. Nor did the *Rht-D1b* comparative controls have smaller cells (width or length) than either DHLs or the *Rht8* parent. Wenzel *et al*. (1997*b*) similarly found no consistent correlation between leaf length and cell number or length in dwarf versus slender barley mutants. Background genetic effects independent of the *Rht* genotype may have accounted for the similar average cell dimensions and leaf size of the DHLs and the *Rht-D1b* comparative controls. The use of near-isogenic *Rht* lines by Keyes *et al*. (1989), Hoogendoorn *et al*. (1990) and Tonkinson *et al*. (1995) would have reduced background genetic effects in their studies on leaf anatomy of GA-insensitive semi-dwarf wheats. Furthermore, the GA-insensitive controls grown here, 'Amery', 'Stiletto' and 'Westonia', are among the most vigorous of the Australian GA-insensitive, semi-dwarf wheats, and have been bred and extensively cultivated in the Mediterranean-type, southern and western regions of Australia's cropping belt. In these regions of winter-dominant rainfall, early vigour is an important, yield-enhancing trait (Botwright *et al*., 2002; Condon *et al*., 2002).

DHLs

There was large genotypic variation for the components of seedling vigour and average cell characteristics among the DHLs in experiments 1 and 2. Yet the contrast between semi-dwarf *Rht8* versus tall *rht8* DHLs revealed no difference in the size of leaf 2 nor in average cell characteristics. These characteristics were therefore independent of plant height. These observations contrast to the known effects of the GA-insensitive *Rht-B1b* and *Rht-D1b* genes, which not only reduce plant height (Allan *et al*., 1961), and coleoptile length (Allan, 1989), but also cause reductions in seedling leaf length and width (Rebetzke and Richards, 1999). Consequently, the use of semi-dwarf *Rht8* wheats in a breeding program would allow for selection of lines with long coleoptiles (Rebetzke *et al*., 1999) and larger leaves (Ellis *et al*., 2004), for both improved stand establishment and early vigour.

Correlations of leaf and average cell size parameters across DHLs revealed a strong and positive relationship between leaf width and cell files. Similarly, leaf length was positively correlated with average cell number per file, although this relationship is biased as the average cell number per file was derived from the leaf length divided by the average cell length. Further quantitative data on the number of cells per file is required to confirm the relationship with leaf length. The DHLs were more similar in early vigour and average cell characteristics to their semi-dwarf, *Rht8* parent, 'Chuan-Mai 18', than their tall, *rht8* parent, 'Vigour18', but selection for greater vigour may be achieved if greater numbers of cell files can be selected, potentially with wider cells. In some DHLs, moderate gains in leaf length above that of the relatively short-leaved *Rht8* parent were achieved by increasing cell number of three of the four cell types, with an increase in cell length only observed in the long cells.

In contrast to these associations, there was no correlation between leaf and cell width, nor between leaf and cell length. These observations contrast with GA-insensitive wheat and barley genotypes where cell length, and not cell number, determines leaf length (Keyes *et al*., 1989; Hoogendoorn *et al*., 1990; Wenzel *et al*., 1997*b*). The lack of correlation between leaf and cell length among the DHLs when genotypic differences existed for leaf length indicated that long leaves were achieved by longer cells in some DHLs, and greater cell numbers in others. Even more vigorous DHLs, combining wider and longer leaves, may have been excluded by the need to constrain the number of DHLs to twelve because of the time-consuming nature of the measurements of cell dimensions. Subsequent early vigour screening of all 190 DHLs has identified a number of vigorous *Rht8* lines that could be used to further clarify the relationships between leaf and cell length, and leaf and cell width.

CONCLUSIONS

In conclusion, we have shown that variation in early vigour, and cell number and size is independent of plant height among a set of semi-dwarf, *Rht8* and tall, *rht8* sister wheat lines. This contrasts to the known effect of the GA-insensitive *Rht-B1b* and *Rht-D1b* dwarfing genes on early vigour and cell size in wheat. In this study, more cell files across the leaf blade contributed to wider leaves and greater early vigour. The retrieval of individuals containing more cell files of greater cell width indicates the opportunity for selecting wheats of even greater vigour in a breeding program targeting greater leaf area development.

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