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Agronomic assessment of the durum *Rht18* dwarfing gene in bread wheat

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ABSTRACT

The wheat Green Revolution *Rht-B1b* and *Rht-D1b* dwarfing alleles are associated with increased grain yields but also with reduced early growth and seedling emergence, especially if sowing conditions are unfavourable. The gibberellic acid-responsive, mutagen-derived *Rht18* dwarfing gene was backcrossed from durum wheat (*Triticum turgidum* subsp. *durum* L.) cv. Icaro into tall bread wheat (*Triticum aestivum* L.) cv. Halberd using phenotypic selection for reduced plant height. The *Rht18* allele was confirmed among homozygous BC_1F_2-derived, $F_{5:7}$ recombinant inbred lines by using a chromosome 6AS-linked, microsatellite molecular marker (*Xwms4608*), and then assessed for agronomic performance across multiple field sites ranging in yield from 3.6 to 6.4 t/ha. The *Rht18*-containing lines were significantly ($P < 0.05$) shorter in height (−24%) and reduced in plant lodging (−51%) compared with tall sister lines. Reductions in plant height were associated with significant increases in grain yield (+16%), reflecting increases in grain number (+21%), number of spikes (+7%) and number of grains per spike (+12%). Coleoptile length, early shoot biomass and ground cover percentage were unaffected by the presence of the *Rht18* dwarfing gene. Comparisons of effects of gibberellic acid-insensitive *Rht-B1b* and *Rht18* on early growth and agronomic performance were assessed separately for a set of 30 BC_5F_6-derived Halberd near-isogenic lines in the field in 2015. Ground cover and coleoptile length were significantly greater for *Rht18* lines, whereas plant height, lodging, harvest index, grain number and yield were similar for *Rht-B1b* and *Rht18* sister lines. Reduced lodging and increased grain number and yield, together with greater coleoptile length, indicate a potentially useful role for *Rht18* in improving wheat performance.

Keywords: coleoptile, dwarf, early vigour, establishment, germplasm, harvest index, lodging, physiology.

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Introduction

Dwarfing genes have been a major driver of improved adaptation and performance with breeding and domestication across all of the major cereals (Hedden 2003). In wheat, the identification and deployment of genes for semi-dwarf stature has promoted the commercial release and global adoption of wheat cultivars with greater yield potential and stability (Perry and D'Antuono 1989; Mathews *et al.* 2006). Selection for reduced crop height remains a key objective of wheat breeding programs worldwide owing to semi-dwarfs being less prone to lodging and producing greater numbers of grain to increase harvest index and crop yields (Hedden 2003).

Despite the availability of >20 dwarfing genes (McIntosh *et al.* 1998; Ellis *et al.* 2004), the deployment of the *Rht-B1b* and *Rht-D1b* dwarfing alleles from the Japanese wheat cultivar Norin 10 has been remarkably widespread in the selection of reduced plant height globally. Indeed, ~70% of the commercial wheat cultivars grown worldwide contain either *Rht-B1b* or *Rht-D1b* (Evans 1998) and both genes were pivotal in yield increases arising out of the Green Revolution (Hedden 2003). However, their value in improving performance in water-limited environments is less clear (e.g. Butler *et al.* 2005; Mathews *et al.* 2006). The *Rht-B1b* and *Rht-D1b* alleles are the most widely deployed of a group of gibberellic acid-insensitive (GAI) dwarfing genes that are unique in reducing

cell-expansion in response to endogenous gibberellins (Hoogendoorn *et al.* 1990; Botwright *et al.* 2005). Reduced cell elongation contributes to reduction in cell length and width without affecting final cell number (Keyes *et al.* 1989; Botwright *et al.* 2005). Reduction in cell size contributes to reductions in internode length, including the peduncle, to affect final plant height (Hoogendoorn *et al.* 1990). This reduction in cell size is ubiquitous in above-ground tissues and contributes to reductions in coleoptile and sub-crown internode lengths, coleoptile tiller frequency and size, and individual leaf area to reduce overall seedling vigour (Allan 1989; Botwright *et al.* 2005; Rebetzke *et al.* 2014).

Establishment is a key phase in the development of high-yielding cereal crops (Rebetzke *et al.* 2007). Optimal plant densities and early leaf area development rely on the seedlings' ability to elongate and emerge with deep sowing, and are important objectives of breeding programs targeting adaptation to water-limited environments (Richards 1992). Improved establishment and early vigour are likely to be more important with climate change and predicted variability with changes in the 'break' for sowing and increasingly greater reliance of deep soil moisture from summer rains (Flohr *et al.* 2021). Greater seedling vigour has also been suggested as a trait for improving weed competitiveness (Coleman *et al.* 2001; Zerner *et al.* 2016) and nutrient uptake (Pang *et al.* 2014; Ryan *et al.* 2015). The constraining influence of GAI alleles such as *Rht-B1b* and *Rht-D1b* on early growth has limited the capacity for breeders developing more-vigorous GAI wheats (Allan 1989; Rebetzke *et al.* 2007, 2014). Several major, gibberellic acid-responsive (GAR) dwarfing genes have been identified with potential to reduce plant height without affecting seedling vigour (Ellis *et al.* 2004; Rebetzke *et al.* 2011, 2012). Many of these genes are available in bread wheat and include *Rht5* (Daoura *et al.* 2014), *Rht8* (Rebetzke and Richards 2000), *Rht12* (Chen *et al.* 2013), and *Rht13* (Rebetzke *et al.* 2011). These genes have been reported to reduce plant height and increase grain yields through greater partitioning of carbon to growing ears, and thereby increase floret fertility to increase grain number (e.g. Rebetzke *et al.* 2012). Reduced height mutants in durum wheat have been reported to contain three GAR dwarfing genes: *Rht14*, *Rht16* and *Rht18* (Haque *et al.* 2011).

The *Rht18* dwarfing gene was first deployed in a semi-dwarf durum cv. Icaro as a direct selection following fast-neutron mutagenesis of the tall wheat Anhinga (Konzak 1988). The *Rht18* gene codes for the GA2oxA9 protein to lower bioactive gibberellic acid content to reduce stem elongation and plant height (Ford *et al.* 2018). There are only two reports of the value of *Rht18* in improving grain yield in wheat, and these represent performance in very small plots in single environments (Yang *et al.* 2015; Tang 2016). The aims of the studies reported herein were to report the transfer of the *Rht18* dwarfing allele from durum into bread wheat and then to assess the effect of this gene on plant height and agronomic performance in backcross-derived, *Rht18*, *Rht-B1b* and tall progeny evaluated in large plots across multiple field environments.

Materials and methods

Transfer of *Rht18* into hexaploid wheat and development of recombinant inbred lines

Hybridisation was undertaken between the *Rht18*-containing, Italian durum wheat cv. Icaro (PI503555) and the tall, non-*Rht18* Australian commercial bread wheat cv. Halberd (PI377885). The F_1 pentaploid seed was harvested, sown and then backcrossed to Halberd to generate BC_1F_1 seeds, which were then self-pollinated to produce BC_1F_2 progeny. Only fertile wheat heads were retained because these were presumed to be fully fertile and therefore genetically hexaploid. This process was repeated for three generations to produce ~120 BC_1F_2-derived, F_5 recombinant inbred lines (RILs). Seeds from individual $F_{4:5}$ plants were sown into rows and a single head was harvested from rows homogeneous for plant height. Harvested heads were threshed and sown into rows in the field for plant height assessment in subsequent generations. About 120 BC_1F_2-derived, $F_{5:7}$ RILs varying for height were identified and bulk harvested. All lines were then sown into a summer nursery for further seed increase. Two inbred lines were discarded based on visual evidence for partial fertility in the summer nursery. For all sowings, the KCD NIL set, containing wheat height near-isogenic lines (NILs: semi-dwarf, *Rht-B1b/D1b*; doubled-dwarf, *Rht-B1d/D1d*; and tall, *Rht-B1a/D1a*), as described in Richards (1992) was sown to aid in height classification.

Genotyping

The simple sequence repeat (SSR) marker *Xwms4608* tightly linked to *Rht18* was used to genotype $BC_1F_{5:6}$ RILs (see Ford *et al.* 2018). In total, 51 RILs homozygous for a 220 bp allele of *Xwms4608* (Halberd allele) averaged mature plant heights of 113 cm, compared with 57 RILs homozygous for the 239 bp Icaro allele averaging 89 cm (−27%) plant height when assessed in a favourable nursery environment.

Agronomic evaluation of all lines

Experiments were undertaken at Temora and Yanco, New South Wales, in 2010, and at Yanco in 2011, 2013, 2014 and 2015. In all experiments, $BC_1F_{5:7}$ RILs were sown at an optimal 3–5 cm sowing depth in 6-m-long, 0.17-m-spaced, 5- or 10-row plots at a seeding rate of ~200 seeds/m^2. Lines were replicated in a row–column experimental design containing partial (p = 1.5) or full (p = 2) replication. Nutrients were supplied at sowing as Starter 15 (14:12.7:11

N:P:S; Incitec Pivot, Melbourne, Vic., Australia) applied at 103 kg/ha and then as commercial urea topdressed at 80 kg/ha at early stem elongation. A pre-sowing irrigation followed by supplemental irrigations as required were supplied to maintain potential growth, and sowing was managed to be free of diseases and weeds with the application of appropriate fungicide and herbicide control measures.

The lines sown included the 108 BC_1-derived *Rht18* RILs and several control entries including: the original parents Icaro and Halberd; and a set of *Rht* F_5-derived sib-based NILs varying for *Rht-B1b* and *Rht-D1b* alleles in CIMMYT-based genetic backgrounds of cvv. Aconchi, Galvez, Kauz, Nesser, Pavon and Seri (Singh *et al.* 2001).

For each plot, phenological development was recorded using the Zadoks development scale (Zadoks *et al.* 1974). Lodging was scored at multiple times throughout grain-filling in each plot (1 = perpendicular to 9 = prostate to the soil surface), and plant height was determined at physiological maturity as the distance from the soil surface to the top of the ear (awns excluded) at three random positions in each plot. At harvest maturity, ~100 culms were hand-cut at ground level using a 40-cm-long quadrat oriented across four rows. Numbers of spikes were counted and samples air-dried at 35°C for 3 days, after which they were weighed and threshed, and grain was weighed. Harvest index calculated as the ratio of grain weight to total aboveground biomass. Plots were end-trimmed to ~5.0 m length and the outside border rows removed before machine harvesting.

Grain size was determined as 100-grain weight from a random sample of grain from the harvest index cuts. Grain number (per area) and grain number per spike were subsequently calculated from grain size and plot yields. For two environments (Yanco 2013 and 2014), percentage shrivelled grain ('screenings'), test weight and grain protein concentration were also determined. Nitrogen (N) yield was calculated from grain protein and grain yield, and assuming a grain protein-N correction factor of 5.7.

Early growth assessment

Three separate experiments were undertaken under field and controlled environment conditions for assessment of early vigour:

(1) Ground cover was estimated on the BC_1F_2-derived RILs from digital images in three environments (Temora in 2010 and Yanco in 2010 and 2011) and encompassing one to three development stages (Z16, Z22 and Z23) representing early, mid and late vegetative growth. Digital images were converted to estimates of ground cover percentage using the vegetation-cover prediction software CanopyCover with parameters iterated before setting to minimise distortion from background soil (Li *et al.* 2010). Normalised difference vegetative index (NDVI) was measured using a GreenSeeker (Trimble, Sunnyvale, CA, USA) reflectance unit at the same time as ground cover images were taken.

(2) Seed from the Yanco 2011 field harvest was sized and sown for early vigour determinations after Rebetzke and Richards (1999). Briefly, good-quality seeds free of any visible damage (particularly shrivelling) and weighing 40–45 mg were obtained for early vigour assessment of each NIL, RIL and parent (Icaro and Halberd). These were sown into wooden seedling trays (600 by 300 by 120 mm) containing a fertile, compost-based potting mix. The experiment was a row–column, partial replicate (p = 1.5) design constraining genotypes from being sown in the same position across replicates. After sowing, trays were placed in an outdoor nursery during the winter until ~3.5-leaf stage, whereupon seedlings were harvested and assessed for numbers of main-stem leaves, and width and length of the first three main-stem leaves. Total leaf area was estimated using a planimeter and dry weights were determined after drying in a 70°C oven for 3 days.

(3) The same seed from the Yanco 2011 field harvest was sized and sown for coleoptile/shoot length determinations after Rebetzke *et al.* (2007, 2014). As for Expt 2, good-quality seeds weighing 40–45 mg were obtained of each RIL, parents Icaro and Halberd, and the CIMMYT height NILs, and sown into wooden trays. Trays were then placed in a darkened growth cabinet with soil temperatures set at a constant 15°C. Seedlings were harvested at 200 degree-days and assessed for length of both coleoptile and the entire shoot (as the entire length from the seed to the tip of leaf 1).

Validation of *Rht18* and *Rht-B1b* dwarfing gene NILs

A set of NILs varying for *Rht-B1b* and *Rht18* was developed in the Halberd background for comparing the effects of the two genes in the field. The *Rht18* NILs were developed from crossing the *Rht18*-containing line HI25M (a selection from the Halberd × 2/Icaro population described above) and genotyping F_1 progeny with the SSR marker *Xwms4608* (see above). This was repeated five times before single-seed descent to generate 15 BC_5F_6-derived *Rht18*-containing NILs. Halberd was also crossed to the *Rht-B1b*-containing cv. Lang and backcrossed five times with selection of *Rht-B1b* containing F_1 progeny using the KASP (Kompetitive allele specific PCR) *Rht-B1b* marker (after Ellis *et al.* 2004). Fifteen BC_5F_6-derived NILs were then developed following single-seed descent. All 30 lines and the tall recurrent parent Halberd were grown under favourable conditions in the glasshouse, and mature seed was harvested before threshing and sowing into deep wooden seedling trays for

assessment of coleoptile length (as above). These same 15 *Rht-B1b* and *Rht18* NILs, and recurrent parent Halberd, were sown in a partial-replicated (p-rep = 1.5), row–column experimental design at the Yanco Managed Environment Facility (Rebetzke *et al.* 2013) in 2015 only. Plots were grown in rainfed and irrigated treatments, with diseases and nutrition managed to maximise crop growth. Sowing depth was confirmed at 4 cm (data not shown). Measurements were made at Z13 and Z14 of NDVI, and at maturity of plant height, number of spikes, grain yield, total biomass, harvest index (as grain yield/total biomass), grain size, grain number, grain protein concentration and N yield.

Statistical analyses

Data were analysed statistically after first checking for normality and error variance heterogeneity across environments. For lodging, the sampling date representing the largest lodging-score mean was used in statistical analysis. All data were analysed as measured except ground cover percentage, which was arcsine transformed prior to statistical analysis. Thereafter, a two-stage, mixed model approach was employed (Cullis *et al.* 1996) using the spatial models procedures in GENSTAT 14th Edition (VSN International, Hemel Hempstead, UK). Each environment was analysed separately with the best spatial models being determined after first fitting the experimental design and then modelling the residual variation with autoregressive row and column terms. Significant spatial effects were then identified, and residuals assessed before determinations were made as to the need for fitting of other (e.g. linear) effects (Cullis *et al.* 1996). The best linear unbiased estimates (BLUEs) for each environment were then used as inputs in the subsequent cross-environment analyses (after McIntosh 1983).

Analysis of the effect of each dwarfing gene on agronomic performance was assessed using: (*a*) a *t*-test for testing dwarf vs tall allele(s) in the CIMMYT-derived *Rht-B1b* and *Rht-D1b* semi-dwarfs with their corresponding CIMMYT tall NILs, and Halberd-derived *Rht18* semi-dwarf RILs with their corresponding tall siblings; and (*b*) a Dunnett's test for comparisons of the Halberd-derived dwarfing gene NILs with tall recurrent parent M808S and Halberd, respectively. Genetic correlations were estimated for plant height and selected traits after Holland (2006). Unless otherwise stated, statistical significance under all null hypotheses of no entry or dwarfing gene difference was at $\alpha = 0.05$.

Results

Weather conditions

Experiments experienced good in-crop rainfalls ranging between 279 and 410 mm across years. Rainfall was particularly high in 2010 and 2013, exceeding long-term averages for the Yanco site (Fig. 1). Years 2011 and 2014 experienced good rainfall before or early in crop growth but were drier from flowering onward, in turn requiring up to 90 mm irrigation during grain-filling. Temperatures were reasonably consistent across years except for above-average temperatures during flowering and grain-filling in 2015 (Fig. 1).

Dwarfing gene contrasts

Halberd-derived Rht18 and tall RILs, and CIMMYT dwarfing NILs

Agronomic performance. Environments in each site × year combination were considered favourable, with grain yields exceeding an average 5 t/ha in all but one environment (Table 1). Average plant height varied significantly among environments, ranging from 90 to 108 cm, and with ranges in plant height across lines within environments of up to 80 cm (data not shown). Grain number and size were commonly large, consistent with the commonly wet and mild conditions experienced in the sampled environments. One site (Temora 2010) was sown to all lines but they were not harvested owing to prolonged rainfall during harvest damaging the mature grain. Notwithstanding, data for plant height and lodging score were collected (Table 1) and have been included in statistical analyses of these traits (Tables 1 and 2).

Genotypic variation was large and statistically significant for plant height measured across all entries (Table 2, Fig. 2). Genotype × environment interaction was small relative to among-genotype genetic variance (data not shown), contributing to high entry-mean repeatabilities for plant height across experiments. Accordingly, the average rank correlation for plant height across environments (r_g) was high at 0.89. The range in genotype means for plant height across all environments was 78–133 cm (Fig. 2). Lines containing the 6AS molecular marker linked to *Rht18* were significantly shorter (−24%) than their tall *rht18* counterparts, and were commonly as short as, or shorter than, *Rht-B1b*- and *Rht-D1b*-containing CIMMYT NILs grown in the same experiments (Fig. 2, Table 2). The shortest *Rht18*-containing lines were significantly shorter than the *Rht18* donor Icaro (cf. Fig. 2 and Table 2) (the two shortest *Rht18* lines were 78 and 81 cm), whereas the tallest lines were significantly taller than the standard height parent Halberd (the two tallest lines were 131 and 132 cm in height). Reduction in plant height with *Rht18* was associated with a significant reduction in (stem) lodging (Table 2), with the shortest *Rht18*-containing lines producing among the highest standability scores in the study (data not shown).

Lines containing the *Rht18*-linked marker had on average significantly greater grain yield across all environments than their tall siblings (Table 2). This increase in yield largely

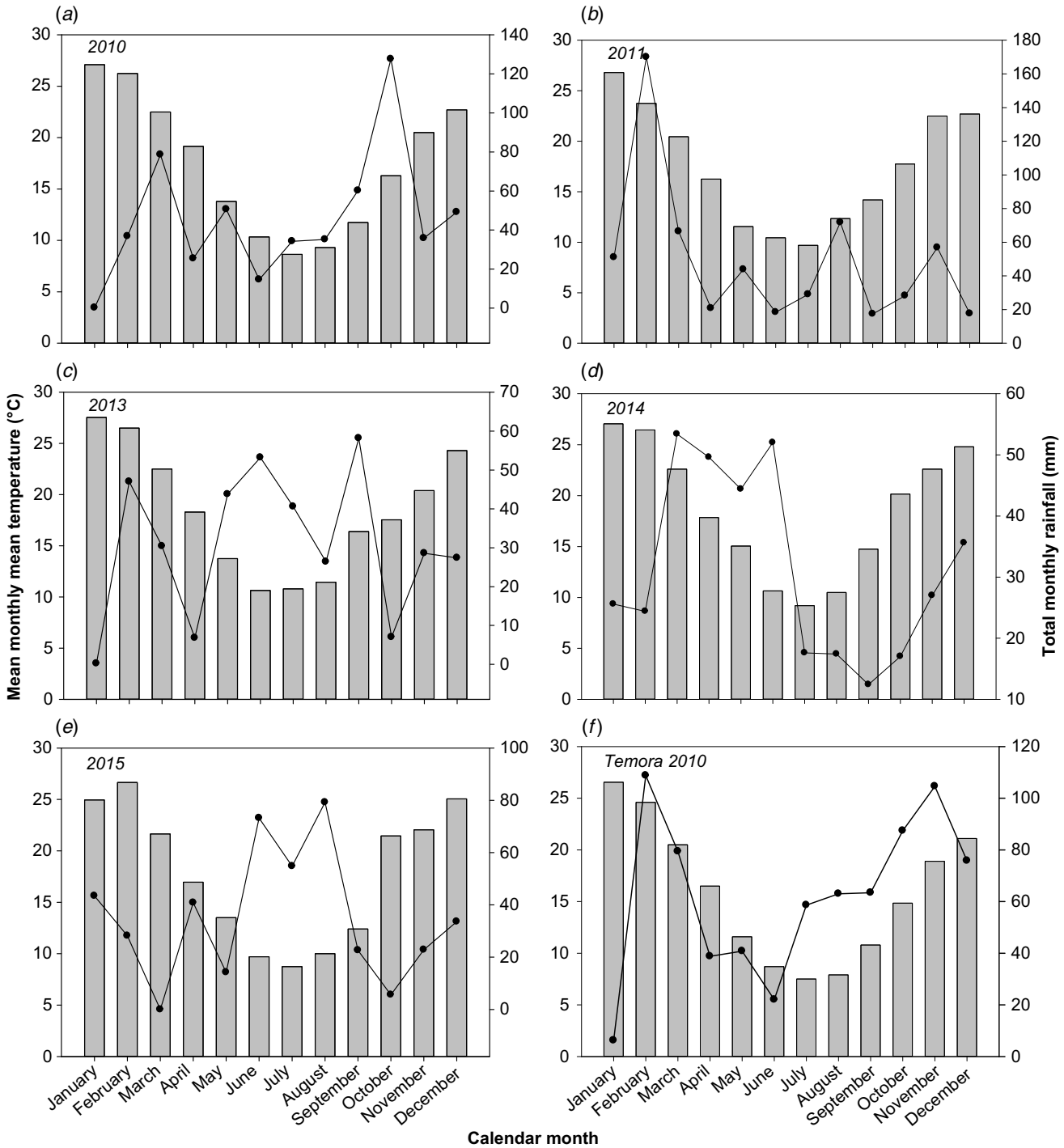

Fig. 1. Mean monthly mean temperature (bars) and total monthly rainfall (line) at Yanco and Temora NSW for years 2010–15 under study.

reflected significant increases in harvest index with little change in total biomass. Anthesis date was similar for semi-dwarf and tall lines (<1 day difference), and grain number was larger (+21%) in lines containing the *Rht18* dwarfing allele (Table 2). Number of spikes and number of grains per spike were greater for lines containing the dwarfing *Rht18* allele (+7% and 12%, respectively), and grain size was significantly smaller (−4%) for semi-dwarf lines (Table 2).

Compared with the tall parent (Halberd), the significantly reduced grain yields of the tall BC_1 derivatives (Table 2) reflects their greater heights and reduced harvest index, and their reduced proportion of Halberd 'adaptation' alleles

Table 1. Environment means for agronomic traits measured on dwarfing gene Halberd BC_1-derived (*Rht18*/*rht18*) RILs and CIMMYT (*Rht-B1b*/*D1b* and *Rht-B1a*/*D1a*) NILs.

Environment	Plant height (cm)	Grain yield (t/ha)	No. of grains per m^2	Grain weight (mg)	Lodging score (1–9)
Temora 2010	90	–[A]	–[A]	–[A]	3.13
Yanco 2010	96	6.43	17 326	37.1	3.83
Yanco 2011	102	6.21	16 244	39.7	3.19
Yanco 2013	97	5.89	14 984	39.3	3.91
Yanco 2014	108	6.44	14 859	43.5	3.95
Yanco 2015	95	4.97	12 474	39.8	7.43
Av. s.e.d.	1	0.10	419	0.08	0.03

[A]Not harvested owing to extreme weather damage of the grain.

Table 2. Genotype group means for plant height and agronomic performance measured on dwarfing gene Halberd BC_1-derived (*Rht18*/*rht18*) RILs and CIMMYT (*Rht-B1b*/*D1b* and *Rht-B1a*/*D1a*) NILs evaluated across multiple environments.

Dwarfing gene NIL/RIL/entry	Height class	Plant height (cm)	Grain yield (t/ha)	Total biomass (t/ha)	Harvest index	No. of grains per m^2	No. of spikes per m^2	Grain weight (mg)	No. of grains per spike	Days to anthesis	Mean lodging score (1–9)
Halberd RILs											
Rht18 (n = 57)	Semi-dwarf	96	6.23	16.5	0.378	17 756	438	35.1	40.5	135	2.22
rht18 (n = 51)	Tall	127	5.38	16.6	0.323	14 680	403	36.6	36.3	133	4.56
	% Change	−24**	+16*	−0.6n.s.	+17*	+21*	+7*	−4.0*	+11.9*	+1.5n.s.	−51**
CIMMYT NILs											
Rht-B1b/*D1b*	Semi-dwarf	97	6.28	15.1	0.417	14917	422	42.1	35.1	128	2.49
Rht-B1a/*D1a*	Tall	124	5.47	14.7	0.372	12 493	439	43.8	28.5	128	5.13
	% Change	−22**	+15**	+2.2n.s.	+12**	+20*	−4n.s.	−3.9*	+23.2**	+0.3n.s.	−52**
Controls											
Halberd (parent)	Tall	121	5.82	17.7	0.328	13 403	443	43.4	30.3	130	6.11
Icaro (parent)	Semi-dwarf	86	5.23	14.4	0.363	10 648	376	49.1	28.3	127	3.46
Av. s.e.d.		3	0.33	1.7	0.029	986	44	0.1	2.5	2	0.46

Rht-B1b and *Rht-D1b* semi-dwarfs include all GAI dwarfing NILs, and *Rht-B1a* and *Rht-D1a* talls include all GAR NILs in the CIMMYT NIL sets.
*P < 0.05; **P < 0.01; n.s., not significantly different (P > 0.05): for comparison between semi-dwarf and tall means.

(~75%). Further, unlike the substantial testing and selection involved in the development of the older but successful cultivar Halberd, these tall progeny lines were random samples from a large genetic population selected only for plant height and not for grain yield or any other agronomic attribute.

Presence of the *Rht-B1b* and *Rht-D1b* dwarfing alleles was associated with significant height reduction in the CIMMYT-based NILs (Table 2). Differences in height between *Rht-B1b* and *Rht-D1b* NILs were small and, in most cases, not statistically significant (data not shown). In the CIMMYT NILs, the GAI dwarfing alleles were associated, on average, with a 22% reduction in plant height. Grain yield was significantly greater for *Rht-B1b*- and *Rht-D1b*-containing NILs, with differences in grain yield being comparable with yield differences in the Halberd-derived *Rht18* lines (Table 2). Greater yield was associated with significantly higher harvest index determined largely though increases in grain number, and particularly number of grains per spike. Total biomass was unchanged between semi-dwarf and tall CIMMYT NILs.

Physical grain quality. The *Rht18* dwarfing gene was associated with a small but non-significant (P > 0.05) reduction in average grain protein concentration compared with tall, non-*Rht18* RILs (Table 3). When coupled with the increased grain yield of *Rht18*-containing lines, N yield was significantly greater for semi-dwarf progeny. Grain protein concentration was significantly lower for the GAI, semi-dwarf CIMMYT NILs; however, their greater yield contributed to increased N yield compared with the CIMMYT tall NILs. Screenings percentage was significantly greater and test weight smaller for *Rht18* than *rht18* RILs (Table 3). Consistent with *Rht18* RILs, test weight was significantly smaller for *Rht-B1b* and *Rht-D1b* CIMMYT-derived NILs. This small difference in screenings between classes of

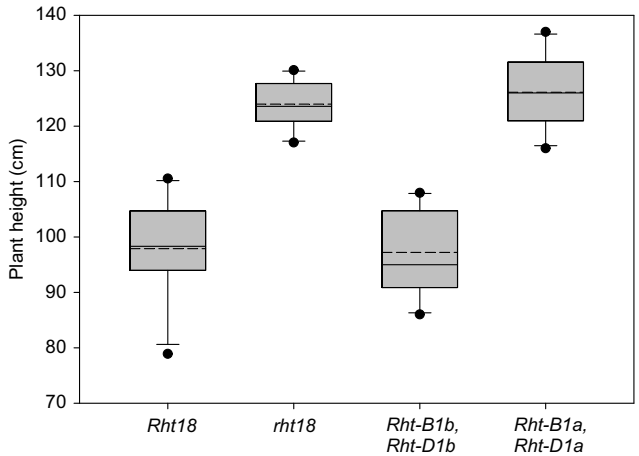

Fig. 2. Overall mean and distribution of maturity plant height for the Halberd × 2/Icaro *Rht18* and *rht18* RILs, and both *Rht-B1b* and *Rht-D1b* semi-dwarf and corresponding *Rht-B1a and Rht-D1a* tall CIMMYT NILs assessed across multiple experiments representing different sites and years. Dots represent extreme line values, whiskers the 5th and 95th percentiles, boxes the 25th and 75th percentiles, and solid and dashed lines the median and mean, respectively, for each population × dwarfing gene class.

CIMMYT NILs may reflect the greater average grain weight of both the semi-dwarf and tall CIMMYT NILs (Table 2).

Coleoptile length. Repeatabilities were high for coleoptile (85 ± 7%) and shoot (78 ± 9%) lengths, respectively, for lines assessed in the controlled environment. The tall, long-coleoptile parent Halberd produced significantly ($P < 0.01$) longer coleoptiles and shoots than the *Rht18* donor Icaro (Table 4), with Halberd's greater length consistent with its longer coleoptile size in previous studies (e.g. Rebetzke *et al.* 2007) and with the size of *Rht-B1a* and *Rht-D1a* tall CIMMYT NILs in the same growth cabinet experiment (cf. Fig 3 and Table 4). In turn, the coleoptiles and shoots of Icaro were longer than those of the *Rht-B1b* and *Rht-D1b* CIMMYT NILs (cf. Table 4 and Fig. 3). The range in coleoptile and shoot lengths was similar for lines with and without the *Rht18*-linked marker (Fig. 3). The range was commonly large for both genotype classes with evidence for transgressive segregation of both shorter and longer shoots. Mean coleoptile and shoot lengths were similar and not statistically different for short *Rht18* and tall genotypes (Table 4).

Early vigour. The influence of *Rht18* on early growth was assessed in RILs phenotyped in both field and controlled nursery environments in Canberra. Parent variety Halberd produced significantly longer and wider seedling leaves than Icaro to increase total leaf area per plant at the 3.5-leaf stage (Table 4). This contrasted with their progeny, where *Rht18* lines had greater mean leaf width than *rht18* lines but were not statistically different for leaf length, plant leaf area or biomass. Numbers of leaves were similar for tall and *Rht18*-containing lines, but the latter produced significantly more tillers at sampling (1.92 and 1.78 tillers for *Rht18* and *rht18* lines, respectively) (data not shown).

Multiple field observations were made of ground cover percentage and NDVI representing different seasons and sites and sometimes multiple growth stages (Fig. 4). The genetic correlation (±s.e.) of ground cover and NDVI was 0.88 (±0.04) at Yanco and 0.85 (±0.04) at Temora at the 5.0-leaf stage, and the pooled genetic correlation across sites was 0.86 (±0.03). The strong correlations reflected the consistent performance of lines for both characteristics across sample dates and environments (Fig. 4). Indeed, genotype × environment and genotype × sample date interactions were small relative to the large genetic variance for ground cover and NDVI (data not shown).

Averaged across all environments, ground cover was not different between *Rht18* and tall lines, whereas NDVI was significantly greater for tall genotypes, although the difference was small (0.321 and 0.332 for *Rht18* and tall lines, respectively). Despite the differences between

Table 3. Genotype group means for grain quality characteristics measured on dwarfing gene Halberd BC_1-derived (*Rht18*/*rht18*) RILs and CIMMYT (*Rht-B1b*/*D1b* and *Rht-B1a*/*D1a*) NILs evaluated across multiple environments.

Dwarfing gene NIL/RIL	Height class	Grain protein concentration (%)	Nitrogen yield (kg/ha)	Test weight (kg/L)	Screenings (%)
Halberd RILs					
Rht18 ($n = 57$)	Semi-dwarf	12.29	127	80.8	2.54
rht18 ($n = 51$)	Tall	12.42	107	81.6	1.47
	% Change	−1.2n.s.	+18.7**	−1.0*	+72.8*
CIMMYT NILs					
Rht-B1b/D1b	Semi-dwarf	12.18	123	78.1	0.94
Rht-B1a/D1a	Tall	12.47	110	79.3	1.20
	% Change	−2.3*	+12.1**	−1.5*	−21.7*

Rht-B1b/D1b semi-dwarfs include all GAI dwarfing NILs, and *Rht-B1a/D1a* talls include all GAR NILs in the CIMMYT NIL sets.
*$P < 0.05$; **$P < 0.01$; n.s., not significantly different ($P > 0.05$): for comparison between semi-dwarf and tall means.

Table 4. Genotype means for parents (P) and semi-dwarf (*Rht18*) and tall (*rht18*) BC_1-derived RILs for early vigour characteristics measured in the field, and in growth cabinets and outdoor nurseries.

Entry	Field environments			Outdoor nursery						Growth cabinet	
	Height class	Ground cover (%)	NDVI	No. of leaves	Mean leaf width (mm)	Leaf 1 length (mm)	Leaf 2 length (mm)	Total leaf area (cm^2)	Shoot dry weight (mg)	Coleoptile length (mm)	Shoot length (mm)
Rht18 ($n = 57$)	Semi-dwarf	24.2	0.321	3.63	4.13	87.1	99.7	12.8	369	122	272
rht18 ($n = 51$)	Tall	26.5	0.332	3.58	3.91	88.2	100.4	12.6	381	124	383
	% Change	+8.7n.s.	+4.0*	−1.4n.s.	−5.6*	+1.3n.s.	+0.7n.s.	−1.6n.s.	−3.2n.s.	+1.6n.s.	+3.9**
Icaro (P)	Semi-dwarf	20.8n.s.	0.249*	3.64*	4.01*	84.1*	95.9*	11.6*	327*	113**	267**
Halberd (P)	Tall	23.6	0.278	3.31	4.21	88.4	101.3	13.5	386	129	286

Ground cover and normalised difference vegetative index (NDVI) means were calculated from early assessments (Z14–Z15) at Temora and Yanco in 2010 and 2011. *$P < 0.05$; **$P < 0.01$; n.s., not significantly different ($P > 0.05$): for comparison between semi-dwarf and tall means.

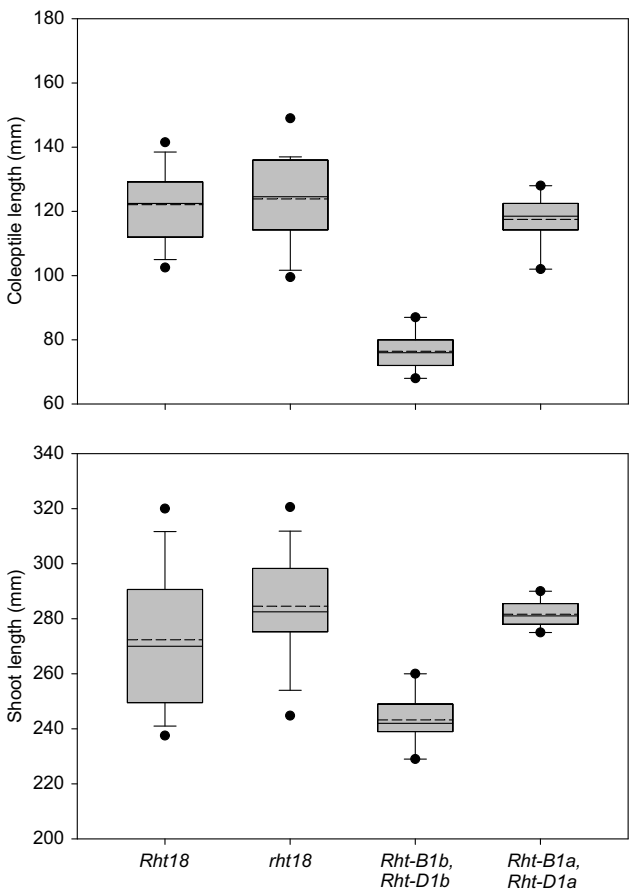

Fig. 3. Overall mean and distribution of coleoptile and shoot lengths for Halberd × 2/Icaro *Rht18* and *rht18* RILs, and both *Rht-B1b* and *Rht-D1b* semi-dwarf and corresponding *Rht-B1a and Rht-D1a* tall CIMMYT NILs assessed at 15°C soil temperature. Dots represent extreme line values, whiskers the 5th and 95th percentiles, boxes the 25th and 75th percentiles, and solid and dashed lines the median and mean, respectively, for each population × dwarfing gene class.

genotype classes, the overlap in individual genotype values was large for both ground cover and NDVI scores (Fig. 4), highlighting that more vigorous *Rht18* lines could be readily identified. Indeed, the two best performing lines for ground cover and NDVI were reduced in height and contained the *Rht18* gene (data not shown).

Halberd-derived Rht-B1b and Rht18 NILs

A high water-limited grain yield potential of 5.0 t/ha was observed for the field assessment of the BC_5 *Rht-B1b* and *Rht18* Halberd NILs at Yanco in 2015 (Table 5). Grain yields for rainfed and irrigated treatments were 3.6 and 5.9 t/ha, respectively. Significant differences were observed for plant height, with the *Rht18* and *Rht-B1b* single-dwarfs reduced by 21% and 19% of the height of the tall recurrent parent Halberd, respectively, whereas the *Rht-B1b* and *Rht18* NILs were themselves not statistically different. Crop lodging score was significantly higher for Halberd, and the *Rht-B1b* NILs had a significantly higher lodging score than the *Rht18* NILs. Harvest index and grain yield of the two semi-dwarf sets of NILs were significantly greater than those of Halberd but were themselves not significantly different (Table 5). The greater grain yields of the semi-dwarf NILs reflected significantly larger grain number, but they were smaller in average grain size. Number of spikes and number of grains per spike were significantly greater for both semi-dwarf NILs than for Halberd. Number of spikes was the same for *Rht-B1b* and *Rht18* NILs, whereas number of grains per spike was larger for the *Rht18* NILs ($P < 0.10$). Maturity biomass was the same for the *Rht-B1b* and *Rht18* NILs. Grain protein concentration was significantly greater for the *Rht18* NILs, but smaller for the *Rht-B1b* NILs, than for Halberd (Table 5). Grain N yield was significantly greater for both *Rht18* (102 kg/ha, +20%) and *Rht-B1b* (94 kg/ha, +11%) than for Halberd, and significantly greater for *Rht18* (+9%) than for *Rht-B1b* NILs.

Number of plants per m^2 was not significantly different for *Rht18* (138 plants/m^2) and *Rht-B1b* (132 plants/m^2) NILs, or recurrent parent Halberd (151 plants/m^2). Early ground cover was assessed as NDVI at crop development stages Z13–14

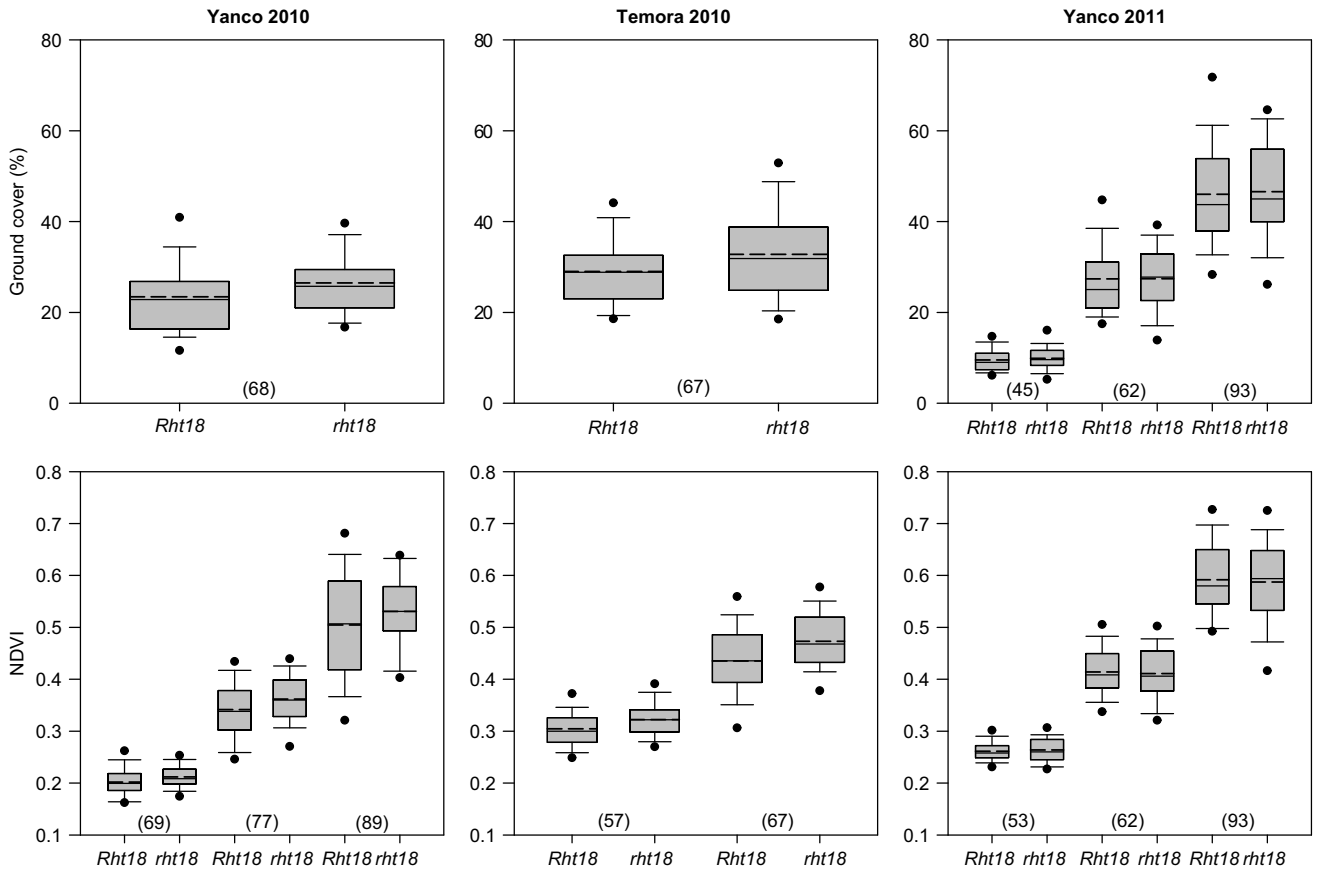

Fig. 4. Ground cover and normalised difference vegetative index (NDVI) for the Halberd × 2/Icaro *Rht18* and *rht18* genotyped RILs assessed in the field at Temora in 2010 and Yanco in 2010 and 2011. Values in parentheses are the days after sowing at which sampling occurred. Dots represent extreme line values, whiskers the 5th and 95th percentiles, boxes the 25th and 75th percentiles, and solid and dashed lines the median and mean, respectively, for each character.

Table 5. Dwarfing gene means for agronomic traits in a field sowing of Halberd-derived *Rht-B1b* and *Rht18* BC_5-derived NILs and the recurrent parent Halberd.

Dwarfing gene	NDVI	Coleoptile length (mm)	Plant height (cm)	Lodging score	Grain yield (t/ha)	Maturity biomass (t/ha)	Harvest index	No. of grains per m^2	100-seed weight (g)	No. of spikes per m^2	No. of grains per spike	Grain protein conc. (%)
Halberd (tall)	0.356	126	109	17.1	4.22	13.56	0.317	10419	4.08	339	30.77	11.16
Halberd + *Rht-B1b*	0.327 (−8.1%**)	101 (−20%**)	88 (−19%**)	6.3 (−63%**)	5.07 (+20%**)	13.4 (−0.4% n.s.)	0.377 (+19%**)	12 610 (+21%**)	4.02 (−2%*)	401 (+18%**)	31.42 (+2%n.s.)	10.70 (−4%*)
Halberd + *Rht18*	0.353 (−0.7%n.s.)	128 (+2%n.s.)	87 (−21%**)	1.4 (−92%**)	5.02 (+19%**)	13.7 (+2%n.s.)	0.367 (+16%**)	12 451 (+20%**)	4.03 (−1%*)	380 (+12%*)	32.77 (+7%*)	11.73 (+5%**)
Rht-B1b vs *Rht18*	−0.258**	−27**	1n.s.	4.9*	0.05n.s.	−0.3n.s.	0.100n.s.	159n.s.	−0.01n.s.	21n.s.	−1.35‡	−1.03**

Coleoptile length means are also included for the NILs in a separate growth cabinet assessment. Comparisons for each dwarfing gene for each trait with recurrent parent Halberd are given as percentage difference (in parentheses), and single d.f. orthogonal contrasts are provided for the two dwarfing gene class means. Mean normalised difference vegetative index (NDVI) was sampled at growth stages Z13 and Z14.
‡$P < 0.10$; *$P < 0.05$; **$P < 0.01$; n.s., no statistical difference at $P = 0.10$: for comparisons between dwarfing gene group.

(Table 5), with *Rht18* NILs being similar in NDVI to recurrent parent Halberd, whereas both *Rht18* lines and Halberd were significantly more vigorous than the *Rht-B1b* NILs. Consistent with NDVI estimates, coleoptile lengths were similar for *Rht18* and Halberd, and both were significantly longer than for *Rht-B1b* NILs.

Discussion

The *Rht18* dwarfing gene had been deployed previously in commercial durum cv. Icaro where it performed well in producing higher grain yields and improved grain quality (Konzak 1988). In our own unpublished glasshouse and field studies, comparisons between Icaro (*Rht18*) and both cvv. Casteloporziano (*Rht14*) and Edmore M1 (*Rht16*) indicated that Icaro was more extreme in height reduction, consistent with the comparisons reported in Haque *et al.* (2011). Further, genetic studies at CSIRO have highlighted *Rht18* as different from the majority of other dwarfing genes in that reduced plant height was inherited as a dominant trait (and thereby had dominant gene action) in F_1 and subsequent segregating generations (Rebetzke GJ unpubl. data). In turn, physiological mechanisms contributing to reduction in plant height might be different from other height-reducing genes. Indeed, Ford *et al.* (2018) has reported the *Rht18* gene as encoding the GA2oxA9 protein to lower bioactive gibberellic acid content. This differs from the *Rht-B1b* and *Rht-D1b* Green Revolution dwarfing genes, which are DELLA mutants rendering plant cells insensitive to endogenous gibberellins.

Large and repeatable genotypic differences were observed for plant height, largely reflecting variation due to *Rht18*. The large height range in the progeny encompassed variation in the parents (the *Rht18*-containing Icaro and the tall parent Halberd), with a number of progeny significantly exceeding either parent. The large range reflects combinations of both the *Rht18* major dwarfing gene from Icaro and minor height-reducing alleles contributed from Halberd. The average 24% height reduction in the *Rht18*-containing lines was comparable to the height reductions of *Rht-B1b* and *Rht-D1b* NILs in the CIMMYT NILs (22% reduction) and in the direct height comparison with the Halberd-derived BC_5 *Rht-B1b* NILs. The observed height reduction with *Rht18* was consistent with results reported previously for both GAR (e.g. Loskutova 1998; Rebetzke *et al.* 2012; Daoura *et al.* 2014) and GAI (e.g. Brandle and Knott 1986; Flintham *et al.* 1997; Rebetzke *et al.* 2001; Ellis *et al.* 2002) dwarfing genes.

Variation in plant height among Halberd-derived progeny was strongly genetically correlated with reductions in crop lodging in the RIL population (r_g = 0.63, P < 0.01). Similarly, both the *Rht18*- and *Rht-B1b*-containing Halberd NILs scored significantly lower than Halberd for lodging, whereas the *Rht18*-containing NILs scored significantly lower than *Rht-B1b*-containing Halberd NILs. The reduced lodging with both *Rht-B1b* and *Rht18* was consistent with the influence of height reduction on lodging reported for GAI dwarfing genes elsewhere (Allan 1989). However, the even greater reduction in lodging associated with *Rht18* was quite remarkable given the similarity in plant height with both *Rht-B1b* and *Rht18* NILs (Table 5). The increased grain yield associated with *Rht18* largely reflected increases in harvest index, with little change in aerial biomass. In turn, increases in harvest index and thereby grain yield were largely due to increases in grain number (r_g = 0.84, P < 0.01) through the production of significantly more spikes and greater numbers of grains per spike (i.e. fertility). Increases in grain number were partly compensated by a reduction in average grain size. Increases in harvest index and grain number for *Rht18* were consistent with the *Rht-B1b* Halberd NILs, and consistent with reports elsewhere for *Rht-B1b* and *Rht-D1b* (Fischer and Stockman 1986; Richards 1992; Flintham *et al.* 1997; Butler *et al.* 2005) and the alternative GAR dwarfing genes (e.g. Rebetzke and Richards 2000; Rebetzke *et al.* 2012). In three winter biparental populations, *Rht18* was associated with reductions in height of 12–25% depending on population (Yang *et al.* 2015). Grain yield and mature plant biomass (on a single-plant basis) were reduced in *Rht18*-containing progeny, whereas harvest index was increased relative to the tall parents. Similarly, the influence of *Rht18* on numbers of spikelets and grains per spike and grain size also varied across populations. In turn, the results of both Tang (2015) and Yang *et al.* (2015) must be treated with caution owing to plot type and size (Rebetzke *et al.* 2014).

The *Rht18* dwarfing gene was associated with small but non-significant reductions in grain protein concentration and a significant increase in grain screenings and test weight compared with non-*Rht18* talls in the RIL population. However, the *Rht18*-containing Halberd NILs produced significantly greater grain protein concentration than both the recurrent parent Halberd and the *Rht-B1b*-containing NILs in the 2015 field study. In all cases, the higher yields associated with *Rht18* were associated with greater N yield. Reductions in grain protein concentration have been observed elsewhere for *Rht-B1b* and *Rht-D1b* (e.g. Brandle and Knott 1986; Flintham *et al.* 1997; McCartney *et al.* 2006).

The lines developed and reported herein served as key parents in genetic studies reported by Flohr *et al.* (2021), Ford *et al.* (2018) and Tang (2015), and have been used elsewhere in germplasm development for delivery to breeding programs. In the present study, the *Rht18* dwarfing gene did not reduce seedling leaf area, biomass or coleoptile length compared with either the tall recurrent parent Halberd or tall RIL siblings, and *Rht18* NILs had significantly greater coleoptile length than *Rht-B1b* NILs. Both ground cover and NDVI were marginally smaller throughout vegetative growth for *Rht18* and tall Halberd BC_1-derived RIL progeny, whereas BC_5-derived *Rht18* NILs had greater NDVI than *Rht-B1b* NILs. Vigorous growth and rapid leaf area development are important in achieving a good plant stand and large canopy early in the season. The shorter coleoptile and reduced NDVI of the *Rht-B1b* NILs is consistent with previous reports for GAI semi-dwarf wheats (e.g. Allan 1989; Rebetzke *et al.* 2007, 2014), and the neutral effect of *Rht18* on coleoptile length and early biomass is consistent with previous observations

for other GAR dwarfing genes (e.g. Ellis *et al.* 2004; Addisu *et al.* 2009; Rebetzke *et al.* 2012). In the work of Yang *et al.* (2015), coleoptile lengths of *Rht18* dwarf sibs were, on average, similar to those of tall parents in two populations while being ~20% shorter in a third population. The GAI *Rht-B1b* and *Rht-D1b* dwarfing genes decrease cell elongation to reduce leaf length and shoot biomass (Ellis *et al.* 2004; Botwright *et al.* 2005), whereas *Rht8* and other GAR dwarfing genes do not affect cell elongation and seedling growth relative to tall, non-dwarfing-gene-containing NILs (Ellis *et al.* 2004; Rebetzke *et al.* 2012). Despite the reduction in leaf length, *Rht18* was not associated with any reduction in leaf area in either the field or controlled environment studies, and maintenance of seedling leaf area and biomass was consistent across the two contrasting genetic backgrounds. In the study of Yang *et al.* (2015), coleoptile length was similar for *Rht18* sibs and tall parents, whereas Flohr *et al.* (2021) reported that coleoptiles of *Rht18* BC_3 NILs were ~30% longer and emerged significantly better than their *Rht-B1b* near-isogenic siblings.

In conclusion, the *Rht18* dwarfing allele has been stably transferred from durum to bread wheat to reduce plant height and lodging and increase grain yield across multiple genetic backgrounds. Presence of *Rht18* increases harvest index and grain number to increase grain yield, consistent with the established influence of the Green Revolution *Rht-B1b* dwarfing gene. This increase reflects increases in spike number and increased spike fertility to increase numbers of grains produced in each spike. The *Rht18* gene appears to reduce seedling leaf length but this does not compromise leaf area owing to commensurate increases in leaf width and greater tillering early in development. The chromosomal location of the *Rht18* gene on the A-genome of wheat and access to linked molecular markers should facilitate ready selection of this allele in bread and durum wheat populations targeting improved adaptation to favourable environments. Further studies are needed to confirm that benefits of this allele extend to improved performance in less favourable environments than those sampled in this study and to develop greater understanding of physiological mechanisms underpinning reduction in plant height by this genetically dominant allele.

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