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Agronomic assessment of the wheat semi-dwarfing gene *Rht8* in contrasting nitrogen treatments and water regimes

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ABSTRACT

Reduced height 8 (*Rht8*) is the main alternative to the GA-insensitive *Rht* alleles in hot and dry environments where it reduces plant height without yield penalty. The potential of *Rht8* in northern-European wheat breeding remains unclear, since the close linkage with the photoperiod-insensitive allele *Ppd-D1a* is unfavourable in the relatively cool summers. In the present study, two near-isogenic lines (NILs) contrasting for the *Rht8*/tall allele from Mara in a UK-adapted and photoperiod-sensitive wheat variety were evaluated in trials with varying nitrogen fertiliser (N) treatments and water regimes across sites in the UK and Spain.

The *Rht8* introgression was associated with a robust height reduction of 11% regardless of N treatment and water regime and the *Rht8* NIL was more resistant to root-lodging at agronomically-relevant N levels than the tall NIL. In the UK with reduced solar radiation over the growing season than the site in Spain, the *Rht8* NIL showed a 10% yield penalty at standard agronomic N levels due to concomitant reduction in grain number and spike number whereas grain weight and harvest index were not significantly different to the tall NIL. The yield penalty associated with the *Rht8* introgression was overcome at low N and in irrigated conditions in the UK, and in the high-temperature site in Spain. Decreased spike length and constant spikelet number in the *Rht8* NIL resulted in spike compaction of 15%, independent of N and water regime. The genetic interval of *Rht8* overlaps with the *compactum* gene on 2DS, raising the possibility of the same causative gene. Further genetic dissection of these loci is required.

1. Introduction

Optimising wheat stature is important to maximise yield and the optimum height varies from 70 to 100 cm according to the yield potential of the environment (Fischer and Quail, 1990; Flintham et al., 1997). Shorter plants are more resistant to lodging (Berry et al., 2007) and can have a greater proportion of assimilates partitioned into the grain, thus increasing grain yield due to improved harvest index (Evans, 1998). In other circumstances, reduced height may reduce biomass yield and improvement in harvest index may be insufficient to compensate, such that grain yield could be reduced (Law et al., 1978; Fernandez et al., 2009; Gooding et al., 2012). Therefore, there is a continuing need for improved under-

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Abbreviations: ANOVA, analysis of variance; Y, yield; HI, harvest index; GN, grain number (m^{-2}); SS, spikelet number ($spike^{-1}$); SN, spike number (m^{-2}); HD, heading date; AN, anthesis; 12L, length of the second internode from the top; 13L, length of the third internode from the top; PAR, photosynthetically active radiation; R: FR, red: far-red light reflectance ratio; RCBD, randomised complete block design.

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standing of the genes which reduce height without yield penalty to inform wheat breeders.

The most common sources of semi-dwarfism in wheat are the gibberellin (GA)-insensitive alleles *Rht-B1b* and *Rht-D1b*, which inhibit cell elongation with subsequent height reduction. The reduced cell-size associated with *Rht-B1b* and *Rht-D1b* also decreases coleoptile length and seedling leaf area, which reduces seedling vigour and compromises emergence from deeper sowing (Rebetzke and Richards, 1999; Botwright et al., 2005). Deeper sowing is preferable in hot and dry conditions which increase seedling mortality, such as in Mediterranean environments, or to avoid animal seed-predation (Mahdi et al., 1998; Rebetzke et al., 2001; Brown et al., 2003; Botwright et al., 2005). Deep-sowing (>5 cm) of shorter-coleoptile *Rht-B1b* and *Rht-D1b* wheats can result in poor and delayed seedling emergence, small leaf area and decreased weed competitiveness (Hadjichristodoulou et al., 1977; Trethowan et al., 2001; O'Donovan et al., 2005; Rebetzke et al., 2007). In addition, though *Rht-B1b* and *Rht-D1b* have increased yield potential in high-input conditions, yield reductions have been reported in environments with low fertiliser inputs (Laperche et al., 2008) and under water-limited conditions (Butler et al., 2005; Chapman et al., 2007). Moreover, the yield increasing effect of the *Rht1* semi-dwarfing alleles does not manifest itself in all genetic backgrounds (Ma et al., 2015).

The main alternative to the GA-insensitive dwarfing alleles found in agriculture is the GA-responsive *Rht8* on chromosome 2DS (Korzun et al., 1998; Gasperini et al., 2012). *Rht8* is well-adapted to dry, Mediterranean-like environments (Worland and Law, 1986) as it provides semi-dwarf stature with the benefits of early seedling vigour and a longer coleoptile (Ellis et al., 2004). *Rht8* is found extensively in southern Europe and parts of eastern/central Europe, as well as China and Australia (Asplund et al., 2012). *Rht8* was introduced to Europe in the early 20th century by the Italian wheat breeder Nazareno Strampelli, unknowingly together with the closely-linked photoperiod-insensitive allele *Ppd-D1a* (Salvi et al., 2013). *Ppd-D1a* reduces time to flowering by early development of floral primordia, without the need for long-day exposure. This is advantageous in climates (such as southern Europe) where earlier flowering and maturation avoids late-season drought stress (Kato and Yokoyama, 1992) and high-temperatures at grain fill (Bennett et al., 2012). The height-reducing effect of *Ppd-D1a* is independent of *Rht8* (Borner et al., 1993) thus it is important to dissect away the effects of *Ppd-D1a* from *Rht8* to clarify genetic contributions. *Rht8* is not found in northern European germplasm and has not been tested extensively in the UK, principally due to the linkage with *Ppd-D1a* (Worland et al., 1998) because in a climate with relatively cooler summers, a long vegetative phase coupled with late flowering is favourable to maximise yield (Kato and Yokoyama, 1992).

Previous agronomic assessments of *Rht8*, many confounded by the pleiotropic effects of *Ppd-D1a*, have mainly focused on height with more limited investigation into yield and yield components. *Rht8* decreases height by 7–18% across varying environments and genetic backgrounds (Ellis et al., 2004; Lanning et al., 2012; Rebetzke et al., 2012; Wang et al., 2015). There is some conflict in yield assessments of *Rht8*. Yield increases of 9.7% (Rebetzke and Richards, 2000) and 3.8% (Borner et al., 1993) have been reported. A further study has reported decreased grain number per spike, biomass and yield, but increased grain weight (2%) and harvest index (6%) (Rebetzke et al., 2012). In more recent agronomic assessments, the *Rht8* allele showed no yield advantage over *Rht-B1b* or *Rht-D1b* or a penalty across different environments in a photoperiod-sensitive background (Lanning et al., 2012). In studies with *Rht8* + *Ppd-D1a*, *Rht8* was reported to have little effect on grain number (-1%), but this was linked with earlier flowering (Addisu et al., 2009; Rebetzke et al., 2012). Another study in a winter-wheat *Ppd-D1a* background reported no difference in grains per spike or grain weight in *Rht8* compared with the tall variety, but found a 17% yield penalty and 10% increase in harvest index (Wang et al., 2015).

There is growing need to maintain high wheat yields at lower N availability (Hawkesford, 2014). For this reason, there is an imperative to understand how the action of semi-dwarfing genes differs at contrasting N applications. The use of *Rht8* in alternative management systems, such as organic agriculture with low N inputs, has been tested to a limited extent in a *Ppd-D1a* background (Addisu et al., 2010). This is despite the promising increase in early crop vigour reported with *Rht8* (Ellis et al., 2004), a trait which has been identified as particularly useful in organic contexts in order to promote early nutrient uptake (Wolfe et al., 2008).

There has been limited work to contrast the performance of *Rht8* in irrigated and non-irrigated systems. In an *Rht8* + *Ppd-D1a* background, *Rht8* conferred increased drought tolerance to potted plants at booting, resulting in increased grain per spikelet relative to *Rht-B1b* and *Rht-D1b* (Alghabari et al., 2014). In the field, *Rht8* NILs in a photoperiod sensitive background yielded less than those with *Rht-B1b* and *Rht-D1b* in conditions with late-season drought and temperature stress (Lanning et al., 2012).

A comprehensive agronomic assessment of *Rht8* in a northern European climate in a commercially-relevant wheat background but without confounding effects of *Ppd-D1a* is lacking. Further, the performance of *Rht8* in Mediterranean conditions without the pleiotropic effects of *Ppd-D1a* requires clarification. This gap in knowledge was addressed in this study by comparing two near-isogenic lines contrasting for the *Rht8* allele from an Akakomugi-derived donor (Mara) in a photoperiod-sensitive background, in order to control for genetic background in our assessments. During population development, a compacted spike was observed which was quantified in this study for the first time. A further objective was to assess the trait responses associated with *Rht8* in contrasting irrigation treatments and a range of N fertiliser inputs.

2. Materials and methods

2.1. Plant material

A 2D recombinant inbred line (RIL) in the Cappelle-Desprez background, carrying the Mara (Akakomugi-derived) semi-dwarfing allele at *Rht8* (Korzun et al., 1998) was crossed to Paragon. Paragon is a high-quality, bread-making UK spring wheat, does not contain the GA insensitive *Rht-B1b* or *Rht-D1b* alleles and is photoperiod sensitive. The population was developed to BC_3F_2, producing NILs contrasting for the Akakomugi-derived *Rht8* allele (short phenotype) and wild-type *rht8* allele (tall phenotype). The presence or absence of the *Rht8* introgression was determined using the *Rht8*-flanking microsatellite markers *Xgwm261* and *Xcfd53* (Korzun et al., 1998; Gasperini et al., 2012). The NIL population was multiplied in the field in 2011 and subsequently one *Rht8* NIL and one *rht8* NIL (herein called 'tall') were selected at the BC_3F_3 stage for further multi-environment field experiments. The selection was made on the basis of preliminary height analysis to identify one semi-dwarf line (*Rht8* NIL) and one tall line (*rht8* NIL).

2.2. Genotyping

For marker-assisted selection, DNA was extracted from ground tissue of young leaves using a commercially available kit (Qiagen) according to manufacturer's instructions. DNA samples were tested for the presence of the *Rht8* allele at the *Xgwm261* (192-bp product) and *Xcfd53* (274-bp product) microsatellite loci using the primer

Table 1
Experimental details of the field-trial sites and the traits measured at each location.

Environment	Soil type	Depth (mm)	Density (seeds m^{-2})	Plot size (m)	Design	Reps	Year	Experimental treatments		Measured traits			Yield (t DM ha^{-1})
								N fertiliser (kg N ha^{-1})	Water regime	Yield	Tiller	Dev	
				1 × 1	–	1	2011	–	–	–	–	–	–
							2012	–	–	Y, HI, GN, TGW, GA	S, SS, I	–	8.7
								100 (N2)	–				8.4
							2013	200 (N3)	–	Y, HI, GN,	S, SS, I	HD, AN	
								–	Irrigated (I)	TGW, GA			8.3
								–	Rainfed (R)				
Norwich, UK (52°37′N, 1°10′E)	Sandyclayloam	50	260	1.5 × 4	RCBD	3		40 (N1)	Irrigated (I)				10.7
									Rainfed (R)				
							2014	200 (N3)	Irrigated (I)				
									Rainfed (R)	Y, HI, GN, TGW, GA	S, SS, I	HD	
								40 (N1)	–				10.6
								100 (N2)	–				
								200 (N3)	–				
								40 (N1)	–		S, SS, I	PAR,R:RF	9.0
Reading, UK (51°29′N, 0°56′W)	Sandy loam	50	260	1.92 × 5	Split-plot	5	2014	100 (N2)	–	Y, HI, GN,SN, TGW, GA			
								200 (N3)	–				
Lleida, Spain (41°37′N, 0°35′E)	Loam	40	300	1.26 × 3.5	RCBD	3	2013–14	–	Fullyirrigated	Y, HI, GN, SN, TGW	–	AN	7.0

sequences and amplification conditions as described previously (Korzun et al., 1998; Gasperini et al., 2012). Products were separated by capillary electrophoresis on an ABI 3730 DNA Analyzer (Applied Biosystems) and manual SSR-allele sizing was performed using GeneMapper v4 software (Applied Biosystems).

Seed from the *Rht8* NIL and Paragon was sent to the University of Bristol Genomics facility to be genotyped on the Axiom® 820,000-feature SNP array (Winfield et al., 2015). Genotypic data was received as ‘AA’, ‘AB’, ‘BB’ and ‘NC’ (missing data) calls (Wang et al., 2014).

2.3. *Field experiments*

The *Rht8* and tall NILs were grown along with Paragon in field trials across three locations: two in the UK (Church Farm, Norwich and Sonning, Reading) and one site in north-eastern Spain (Lleida). The details of the sites and experimental design are presented in Table 1, with the traits measured at each location. The NILs were grown over three growing seasons (October to August) in Norwich in 2011–2014; two seasons in Lleida (2012–2014) and one season in Reading (2013–2014). Drilling dates were third week of November 2012 and 2013 in Lleida, 17th October 2013 in Reading, 13th October (nitrogen experiment)/16th October (irrigation experiment) 2012 and 19th October 2013 in Norwich. Plots were separated in Reading by 0.5 m double-width track wheelings, in Norwich by 0.8 m paths and in Lleida by 1 m paths. Experiments in Norwich and Reading included contrasting nitrogen (N) treatments and in Norwich only, contrasting water regimes (Table 1). The experiments in Lleida were irrigated to field capacity and received N fertiliser according to standard agronomic practice. For trials with contrasting N treatments, 40 kg N ha^{-1} (N1) was applied at Zadoks growth stages GS30-31 (Zadoks et al., 1974) and a further dose of N applied at GS34-39 to make up to the required levels for N2 (total 100 kg N ha^{-1}) and N3 (total 200 kg N ha^{-1}). For the irrigation experiments, trickle irrigation was applied using a timer and piping between each row within a plot. In 2013, water was applied from mid-June to July (after stem elongation) five days per week, receiving 15 L m^{-2} day^{-1}. In 2014, irrigation was applied from April to May on 14 occasions receiving approximately 14 L m^{-2} day^{-1}. Field trials were kept weed- and pest-free with products according to standard agronomic practice at each of the locations, with the exception that plant growth regulators (PGRs) were not applied.

2.4. *Climate and day length*

Weather data for Reading and Lleida were recorded at automated meteorological stations at the sites and from a station ~15 km from the Norwich trial site (http://www.buxton-weather.co.uk/weather.htm). Mean temperature was based on the daily minimum and maximum temperatures averaged over the month. The day lengths for the 2013–2014 growing season for Norwich and Lleida were calculated using the maptools package (Bivand and Lewin-Koh, 2015) in R v3.1.1 (R Development Core Team, 2014), using the numeric difference between sunrise and sunset. The day lengths between the two UK sites were presumed to be comparable.

2.5. *Phenotyping and assessments*

Grain yield and yield components were determined at maturity for each plot. In Lleida, grain yield was obtained by harvesting manually and 1 m centrally in each plot. In Reading, the central 1.4 m of each plot was combine harvested for grain yield assessment and for Norwich the entire plot was used. Grain m^{-2} (GA) was calculated from grain yield and thousand grain weight (TGW). Plant height (pH) was measured from soil level to the top of each wheat ear. This was measured on the plot level at maturity from a visually-determined representative tiller per plot in Norwich and Lleida, and in Reading using a rising disc of polystyrene (Peel, 1987). Plot-level pH was used for comparisons between field experiments. The spike number m^{-2} (SN) was calculated from plant populations taken at Reading and Lleida using the mean of three 0.1 m^2 circular quadrats in each plot.

Developmental stages of heading date (HD) at GS57 and anthesis (AN) at 50% emergence from the flag leaf were recorded when 50% of the plants in each plot reached that stage. HD and AN were assessed in thermal time of °C days, by calculating the cumulative temperature from drilling to assessment date. The mean daily temperature was calculated from the minimum and maximum daily temperatures from the weather data. At Reading, the proportionate interception of photosynthetically active radiation (PAR) was measured below the canopy (10 mm above soil level) with a ceptometer (AccuPAR LP-80 Decagon Devices Inc., Pullman, Washington). Red: far-red (R: FR) reflectance ratios were measured pre- (October, March) and post-anthesis (June, July) with narrow angle sensors (SKR 1800, Skye Instruments Ltd, Llandrindod Wells, UK) for 730 and 660 nm positioned 0.8 m above soil level. Prior to harvest, three main tillers from three plants were sampled from each plot in the UK sites only. Tillers were used for assessment of height (total stem length from soil to tip of spike), spike and internode lengths (by extending tillers along a ruler). The height from the stem length was used rather than the plot-level height when measuring the contribution of height components. Tillers were also used to measure yield components: harvest index (ratio of grain weight to above-ground biomass) (HI), spikelet number $spike^{-1}$ (SS) and TGW. Above-ground biomass was measured before threshing the grain. Morphometric measurements (grain area (GA) and TGW) were recorded from threshed grain using 300–400 grains per sample on the MARVIN grain analyser (GTA Sensorik GmbH, Germany). The internode below the spike was defined as the peduncle and the successive internodes as the second and third internodes, respectively. Each internode was measured from the mid-point of the subtending node.

Lodging was measured in Norwich in each plot where any degree of lodging had occurred at approximately GS70 in July 2014, following a storm. Lodging score was calculated using the percentage of the plot area which had lodged multiplied by the angle of lodging (0–90°) (Fischer and Stapper, 1987). Lodging score ranged from 0 to 100, with 0 being no lodging and 100 being total displacement to horizontal across the whole plot.

Spike compactness was quantified using the tiller samples from Norwich and Reading taken before harvest. Spike length and spikelet number $spike^{-1}$ were used to calculate compactness as cm $spikelet^{-1}$.

2.6. Data analysis

Comparisons between NILs were carried out using genotype analyses of variance (ANOVAs) to assess the effects of genotype within treatment combinations. For Lleida, a two-way ANOVA was performed for data across both years, using a treatment structure of year × genotype with block as the random effect. For Norwich, for the N and irrigation trials in 2013 and for N in 2014 a two-way ANOVA was performed with the treatment × genotype (treatment structure) and block as random effect. Residual Maximum Likelihood (REML) analysis was carried out for the irrigation experiment at Norwich in 2014, where the fixed effects were N treatment × water treatment × genotype. The NILs in Reading were compared at different N treatments using a split-plot ANOVA with N as the main plot and genotype the sub-plot. ANOVA and REML were performed using GenStat 16th edition (VSN International). Fisher's least significant difference (l.s.d) test was used to determine significant differences between means at the 0.05 level.

3. Results

3.1. Inter-site comparison

The UK sites had shorter days in winter and longer days in summer relative to Lleida (Supplementary Fig. 1a). The Lleida site was representative of hotter Mediterranean conditions but not water limitation, as plots were irrigated. A range of temperature was encountered in the UK and Lleida (Supplementary Fig. 1b) with Lleida experiencing higher temperatures throughout the reproductive and grain-filling phases. Lleida had a higher temperature range (4 °C to 24 °C in July, when harvest was completed) than the UK (low of 4 °C and high of 18 °C in Reading). The two UK sites had similar climates, differing only slightly in temperature at the end of stem elongation/beginning of grain filling (Supplementary Fig. 1e). Reading was 2 °C hotter than Norwich in June and 1 °C hotter in July (Supplementary Fig. 1b). There was more rainfall at the start of the season in Reading compared to Norwich (Supplementary Fig. 1d) during the vegetative phase followed by a drier latter half of the season in Norwich. UK sites had markedly lower levels of solar radiation (Supplementary Fig. 1c), with half the levels of Lleida in some months. Norwich was the highest yield-potential site with an average yield close to 11 t ha^{-1}, compared to 9 t ha^{-1} in Reading, and 7 t ha^{-1} in Lleida (Table 1).

3.2. Genotyping

To assess the genotypic background and effectiveness of isogenic population development, the *Rht8* NIL was genotyped along with Paragon using the 820,000 feature Axiom® SNP array. A total of ~56,000 SNPs were found between the *Rht8* NIL and Paragon, which was reduced to 6088 SNPs without missing data (data not shown). This indicated a 99.4% homogeneous background, well above the expected theoretical 93.875% homogeneity in the BC_3-derived NILs. Within the identified SNPs, 2% were mapped to chromosome 2DS, where the *Rht8* introgression is located.

3.3. Plant height and associated traits

Comparison of the *Rht8* NIL, tall NIL and Paragon using ANOVA showed that there was a highly significant difference between genotypes in pH in all environments ($P < 0.05$ in 2012, $P < 0.001$ in all other environments) (Table 2). The tall NIL was taller than Paragon in all environments except Lleida in 2014. To mitigate these background genetic effects, trait responses of the *Rht8* NIL were considered relative to the tall NIL. pH of the *Rht8* NIL ranged from 88 to 115 cm (excluding bulking seed in 2011) and was on average 11% shorter than the tall NIL across environments (Table 2). The height reductions were relatively consistent across environments with the exception of the two seasons in Lleida which were the most variable within a single location, with the highest and lowest percentage difference relative to the tall NIL reported (−1 to −22%).

The reduction in pH resulted from shortening the length of internodes across all environments and treatments (Supplementary Table 1). The greatest reduction and greatest number of significant differences between genotypes was in peduncle length (PL). When compared to the tall NIL, PL was shortened by 7–21%, with a mean reduction of 14%. The second (12L) and third (13L) internodes from the top had smaller reductions in *Rht8* relative to the tall NIL, with a mean 11 and 4% decrease across environments, respectively.

These results indicate that *Rht8* conferred a stable and significant height reduction of ~11% relative to the tall NIL, across

Table 2
Mean values for plant height in all environments of the Rht8 NIL, tall NIL and Paragon.

Environment	Year	Experimental treatments		Paragon (control)	Tall	*Rht8*	l.s.d
		N fertiliser (kg N ha^{-1})	Water regime				
Norwich	2011	–	–	68.0	68.5	59.5 (−13%)	–
	2012	–	–	93.4	99.0	87.8 (−11%)	7.6*
		100 (N2)	–	103.7	107.3	94.3 (−12%)	3.2***
	2013	200 (N3)	–	107.7	113.7	98.3 (−13%)	3.2***
		–	Irrigated (I)	108.7	114.0	100.7 (−12%)	3.3***
		–	Rainfed (R)	106.8	112.7	97.3 (−14%)	3.3***
		40 (N1)	Irrigated (I)	103.3	109.9	96.3 (−12%)	4.6***
			Rainfed (R)	100.9	106.4	92.5 (−13%)	4.6***
	2014	200 (N3)	Irrigated (I)	115.4	113.7	104.2 (−8%)	4.6***
			Rainfed (R)	111.2	115.6	102.8 (−11%)	4.6***
		40 (N1)	–	100.9	106.4	92.5 (−13%)	5.0***
		100 (N2)	–	102.8	110.5	96.5 (−13%)	5.0***
		200 (N3)	–	111.2	115.6	102.8 (−11%)	5.0***
Reading	2014	40 (N1)	–	97.5	105.1	93.0 (−12%)	2.9***
		100 (N2)	–	104.9	109.7	100.7 (−8%)	2.9***
		200 (N3)	–	108.2	112.2	103.9 (−7%)	2.9***
Lleida	2013–2014	–	Fully irrigated	111.0	119.0	93.3 (−22%)	7.1***
				119.0	115.3	114.7 (−1%)	7.1***

environments of varying yield potential and climatic conditions. The magnitude of the height difference between the *Rht8* and *tall* NIL was proportionately unaffected by N or irrigation treatment and the total height reduction was principally contributed by the spike, peduncle and second internode from the top.

3.4. Spike compactness

Semi-compact spike morphology was discernible in field plots in the *Rht8* NIL (Fig. 1a) and present in spikes of different spikelet numbers (Fig. 1b). The trait was quantified by dividing SL by the spikelet number spike^{-1} (SS), to achieve a unit of cm spikelet^{-1}. Spike compactness was significantly different ($P < 0.05$ and $P < 0.001$) between the genotypes in every environment and treatment (Table 3). Spike compactness was consistently greater in the *Rht8* NIL than the tall (ranging from 13 to 23% greater), with a mean increase in compactness of 15%. SL closely mirrored the pattern observed in the spike compactness. The *Rht8* NIL consistently had a significantly ($P < 0.001$) shorter spike than the tall NIL across environments, with a mean 15% decrease (Table 3). SS was not significantly different between genotypes in any environment. The results indicate that despite spike compactness being a function of SL and SS, the difference in compactness in the *Rht8* NIL was driven by the reduction in SL, rather than by an increased number of spikelets on the rachis.

3.5. Effects on lodging

In July 2014, a storm with high winds caused root lodging in field plots at ~GS70 at the Norwich site. There was a significant difference in lodging between the NILs ($P < 0.001$), with the *Rht8* NIL having half the mean lodging score (38%) of the tall NIL (74%) at the highest N treatment (N3, Fig. 2). At N1 and N2, the *Rht8* NIL was completely resistant to lodging (Fig. 2).

3.6. Effects on developmental traits

The effects of *Rht8* on developmental traits are often reported in a *Ppd-D1a* background. In the current study in a spring wheat background, developmental traits could be analysed without the earliness conferred by photoperiod insensitivity (Supplementary Table 2). Heading date (HD) was measured in Norwich across two seasons, 2013 and 2014. N treatment had no significant effect in either year on HD and irrigation significantly ($P < 0.001$) delayed HD across all NILs by ~1 calendar day, but only in the 2014 season. Mean HD did not differ significantly (l.s.d. test) between the *Rht8* and tall NILs.

Fig. 1. Compact spike morphology between the *Rht8* NIL and Paragon. Panel *a* shows the Rht8 NIL in the field (right) compared to the more lax spike in Paragon (left); panel *b* shows compacted *Rht8* spikes with the same spikelet number as Paragon (22 spikelets, left; 24 spikelets, right). The scale is cm.

Anthesis (AN) was measured in 2014 in Norwich and in Lleida across the 2013–14 seasons. Overall, different N treatment or water regime had no significant effect on AN (Supplementary Table 2). Significant ($P < 0.05$) differences between the *Rht8* and tall NIL were observed in two of the four treatments in Norwich. At N2 and in rainfed conditions the *Rht8* NIL had a mean delay of 1–1.4% in thermal time to anthesis compared to the tall NIL, which equates

Table 3
Mean values for spike compactness and derivative traits in all environments of the Rht8 NIL, tall NIL and Paragon.

Trait	Environment	Year	Experimental treatments		Paragon (control)	Tall	*Rht8*	l.s.d
			N fertiliser (kg N ha^{-1})	Water regime				
		2012	–	–	0.46	0.48	0.41 (−15%)	0.05*
		2013	100 (N2)	–	0.51	0.51	0.42 (−17%)	0.03***
			200 (N3)	–	0.46	0.47	0.38 (−18%)	0.03***
			–	Irrigated (I)	0.47	0.46	0.39 (−17%)	0.04***
			–	Rainfed (R)	0.47	0.51	0.39 (−23%)	0.04***
	Norwich		40 (N1)	Irrigated (I)	0.46	0.47	0.41 (−13%)	0.02***
				Rainfed (R)	0.44	0.45	0.37 (−19%)	0.02***
Spike compactness (cm spikelet^{-1})		2014	200 (N3)	Irrigated (I)	0.46	0.50	0.41 (−18%)	0.02***
				Rainfed (R)	0.49	0.50	0.42 (−16%)	0.02***
			40 (N1)	–	0.44	0.45	0.37 (−19%)	0.05***
			100 (N2)	–	0.52	0.50	0.41 (−18%)	0.05***
			200 (N3)	–	0.49	0.50	0.42 (−16%)	0.05***
			40 (N1)	–	0.42	0.43	0.37 (−14%)	0.03***
	Reading	2014	100 (N2)	–	0.45	0.45	0.41 (−20%)	0.03***
			200 (N3)	–	0.48	0.50	0.42 (−14%)	0.03***
		2012	–	–	11.2	11.48	10.28 (−10%)	1.70^{NS}
		2013	100 (N2)	–	10.71	10.72	8.81 (−18%)	0.70***
			200 (N3)	–	10.63	10.43	8.83 (−15%)	0.70***
			–	Irrigated (I)	10.65	10.81	8.78 (−19%)	0.80***
			–	Rainfed (R)	10.73	11.23	9.09 (−19%)	0.80***
	Norwich	2014	40 (N1)	Irrigated (I)	10.59	10.44	9.29 (−11%)	1.50***
Spike length (cm)				Rainfed (R)	10.04	9.89	8.10 (−18%)	1.50***
			200 (N3)	Irrigated (I)	10.54	11.42	10.41 (−9%)	1.50***
				Rainfed (R)	11.89	11.50	9.84 (−14%)	1.50***
			40 (N1)	–	10.04	9.89	8.10 (−18%)	0.09***
			100 (N2)	–	11.96	11.21	9.16 (−18%)	0.09***
			200 (N3)	–	11.89	11.50	9.84 (−14%)	0.09***
			40 (N1)	–	9.90	9.90	9.10 (−8%)	0.08***
			100 (N2)	–	10.4	10.40	8.30 (−20%)	0.08***
	Reading	2014	200 (N3)	–	10.9	11.30	10.20 (−10%)	0.08***
		2012	–	–	24.11	23.89	25.00 (+5%)	1.02^{NS}
			100 (N2)	–	20.94	21.00	20.89 (−1%)	1.46^{NS}
		2013	200 (N3)	–	23.11	22.22	23.00 (+4%)	1.46^{NS}
			–	Irrigated (I)	22.61	23.33	22.78 (−2%)	1.57^{NS}
			–	Rainfed (R)	22.83	22.22	23.22 (+5%)	1.57^{NS}
Spikelets (spike^{-1})	Norwich		40 (N1)	Irrigated (I)	23.00	22.00	22.11 (+0%)	2.10^{NS}
				Rainfed (R)	23.11	22.00	22.56 (+3%)	2.10^{NS}
			200 (N3)	Irrigated (I)	23.22	22.89	25.44 (+11%)	2.10^{NS}
		2014		Rainfed (R)	24.33	23.11	23.44 (+1%)	2.10^{NS}
			40 (N1)	–	23.00	22.00	22.11 (+0%)	2.00^{NS}
			100 (N2)	–	23.11	22.67	22.56 (+0%)	2.00^{NS}
			200 (N3)	–	24.33	23.11	23.44 (+1%)	2.00^{NS}
			40 (N1)	–	23.56	22.89	24.89 (+9%)	1.69^{NS}
	Reading	2014	100 (N2)	–	23.22	23.33	23.22 (+0%)	1.69^{NS}
			200 (N3)	–	22.89	22.67	23.56 (+4%)	1.69^{NS}

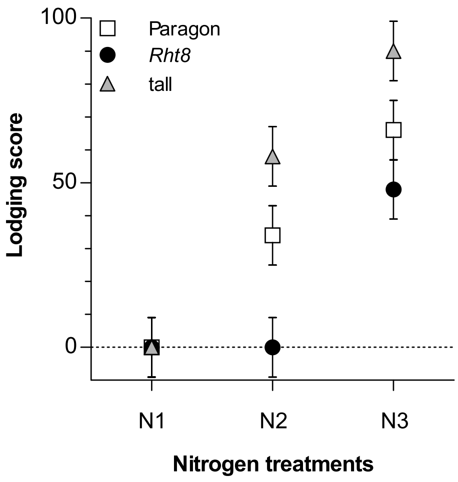

Fig. 2. Effect of nitrogen treatment on lodging in the *Rht8* NIL, tall NIL and paragon. These data are the mean values ± SEM (standard error of the mean).

to 2–2.5 calendar days. In Lleida, there was no difference in AN between the genotypes in either season (Supplementary Table 2).

Light quantity (PAR) and quality (R: FR) at base canopy level were measured in Reading in 2014. Pre-anthesis, there was no difference in R: FR reflectance between the NILs (Supplementary Table 2). From October to March, the R: FR reflectance halved from ~1 to 0.5, reflecting the canopy growth and increase in density which promoted mutual shading among plants. Post anthesis, R: FR reflectance increased with time, reaching ~0.85 by GS85 (end of grain filling). N treatment only had a significant ($P < 0.001$) effect on R: FR reflectance in early–mid July, and increased N reduced R: FR reflectance across all genotypes in equal proportion (treatment × allele $P = 0.8$). The only significant ($P < 0.01$) difference between the *Rht8* and tall NIL occurred on 7th July, with an 18% increase in R:FR reflectance in *Rht8* at the N2 treatment. Over all N treatments at the first time-point post-anthesis, the *Rht8* NIL had a mean 14% increase in thermal time to AN compared to the tall NIL.

Interception of photosynthetically active radiation (PAR) was measured post-anthesis at three time points in July 2014 (Fig. 3). At all time-points, there was a significant ($P < 0.001$) effect of N treatment: PAR interception increased ~5% between incremental N treatments, such that there was ~10% reduction in PAR interception at N1 compared with N3. There was a significant ($P < 0.05$) difference in PAR between NILs, with the mean of the *Rht8* NIL

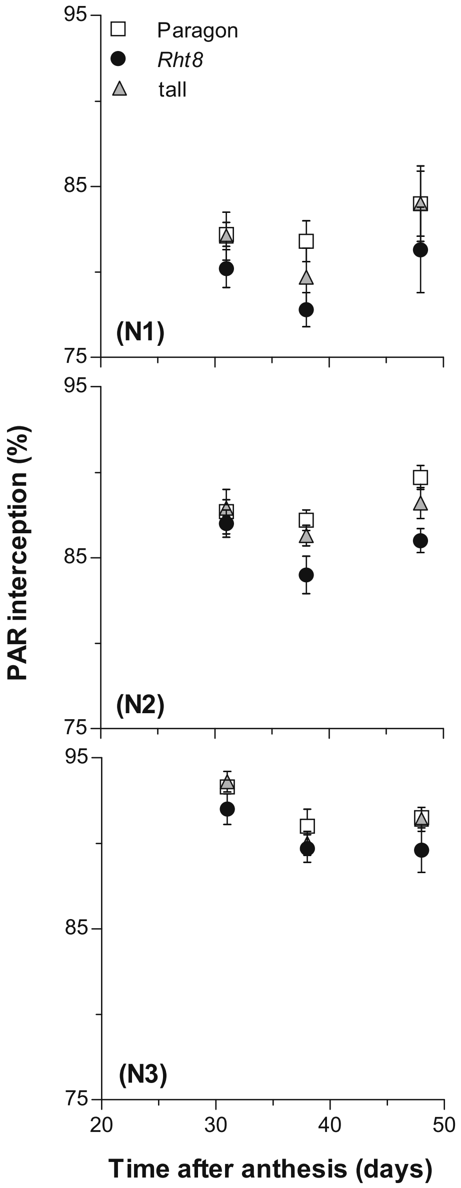

Fig. 3. The effect of N treatment on PAR interception in the *Rht8* NIL, tall NIL and Paragon in Reading 2014. These data are the mean values ± SEM (standard error of the mean).

intercepting less than the tall NIL at each time-point. Furthermore, the differential between the *Rht8* and tall NIL increased with time (−1.4%, −1.5% and −2.3%).

3.7. Grain yield and yield components

To determine if the *Rht8* introgression conferred adaptation to lower input conditions, the NILs were grown in experiments with contrasting N treatments and water regimes. The trials in Lleida were fully irrigated, and this provided the opportunity to observe increased adaptation to high temperature. The temperatures in the Lleida growing season were high relative to the UK (Supplementary Fig. 1b), but were below the 27°–30 °C range at AN which has been used to define 'heat stress' (Semenov et al., 2014). A comprehensive dataset of yields and main yield components in each location is shown in Supplementary Table 3.

In standard UK agronomic conditions (rainfed and high N (N3, 200 kg N ha^{-1})), the *Rht8* NIL conferred a mean yield penalty of −8% across environments, ranging from −4 to −26% relative to the tall NIL (Supplementary Table 3). Exceptions to the yield penalty in the UK were in non-standard agronomic conditions at lower N treatments and in irrigated conditions. In Reading, the yield penalty was overcome at the lowest N input (N1) (Fig. 4a), whereas in Norwich the yield penalty was not observed at N1 or N2 (Supplementary Table 3). Irrigation treatments were conducted in the 2013 and 2014 growing seasons in Norwich. At 200 kg N ha^{-1} (N3), the yield penalty associated with the *Rht8* introgression in rainfed conditions was eradicated by providing irrigation. This result was observed across both years (Supplementary Table 3). At N1, irrigation increased yield proportionally across all genotypes so that the yield penalty in the *Rht8* NIL was maintained.

The yield penalty in the *Rht8* NIL observed in standard UK agronomic conditions was not replicated in the high-temperature Lleida environment. There was a borderline significant ($P = 0.07$) increase in mean yield of the *Rht8* NIL compared to the tall: 10% in 2013 and 16% in 2014 (Fig. 4b).

Grain number m^{-2} (GN) had the greatest number of significant differences between genotypes in the main yield components measured (Supplementary Table 3). There was a highly positive and significant linear relationship ($r^2 = 0.784$, $P < 0.001$) between the difference of the *Rht8* NIL response compared to tall in yield and GN (Fig. 5a). The negative impact associated with the *Rht8* introgression on yield was closely mirrored by a concomitant decrease in GN, averaging −7% in standard agronomic conditions. Where the yield penalty was overcome, in Lleida and at low N levels, the decrease in GN was also eliminated (Fig. 4c, d). The difference in GN between the NILs was not significant in Lleida ($P = 0.1$) with the mean GN of the *Rht8* NIL 2% (2013) and 10% (2014) higher than the tall NIL (Fig. 4d).

Spike number m^{-2} (SN), a component of GN, was obtained in two of the sites: Lleida and Reading. There was a highly significant ($P < 0.001$) reduction in SN in the *Rht8* NIL compared to the tall in Reading (a mean of −20%) at N1 and N3 (Fig. 4e). In Lleida, this reduction was reversed: there was no longer a significant difference in SN between NILs, with the *Rht8* NIL showing a mean 11% increase across both years (Fig. 4f).

Reponses of the *Rht8* NIL relative to tall were smaller in other yield components (Supplementary Table 3). In comparison with GN, there were fewer significant differences between genotypes in TGW, though across environments the relationship between change in yield and change in TGW in the *Rht8* NIL was significant (Fig. 5b, $r^2 = 0.293$, $P < 0.05$). In 2013 at Norwich, the *Rht8* NIL had a significant ($P < 0.05$) mean 5% increase in TGW at N3 and a mean 7% increase in Lleida ($P < 0.05$), but a smaller mean TGW than the tall in all other standard agronomic conditions. The harvest index (HI) was significantly higher in the *Rht8* NIL compared to tall only in 2013 at Norwich (mean 4% increase). However, in the subsequent year, the *Rht8* NIL had a mean7% reduction in HI relative to the tall NIL. Thus this trait did not have a robust response and overall there was no significant relationship between change in yield and change in HI in the *Rht8* NIL compared to the tall (Fig. 5c). There was no relationship between change in yield and change in grain area (GA) in the *Rht8* NIL relative to the tall (Fig. 5d) though there was a significant increase of 3% at N2 and N3 in 2013 in Norwich in the *Rht8* NIL. There were no differences between genotypes in any environment in the number of spikelets spike^{-1} (SS) (Supplementary Table 3).

4. Discussion

This study was carried out to assess the adaptive advantage of *Rht8* in UK-adapted germplasm in northern Europe, without linkage to *Ppd-D1a*, which has confounded some previous reports into *Rht8* in different genetic backgrounds (Addisu et al., 2010; Alghabari

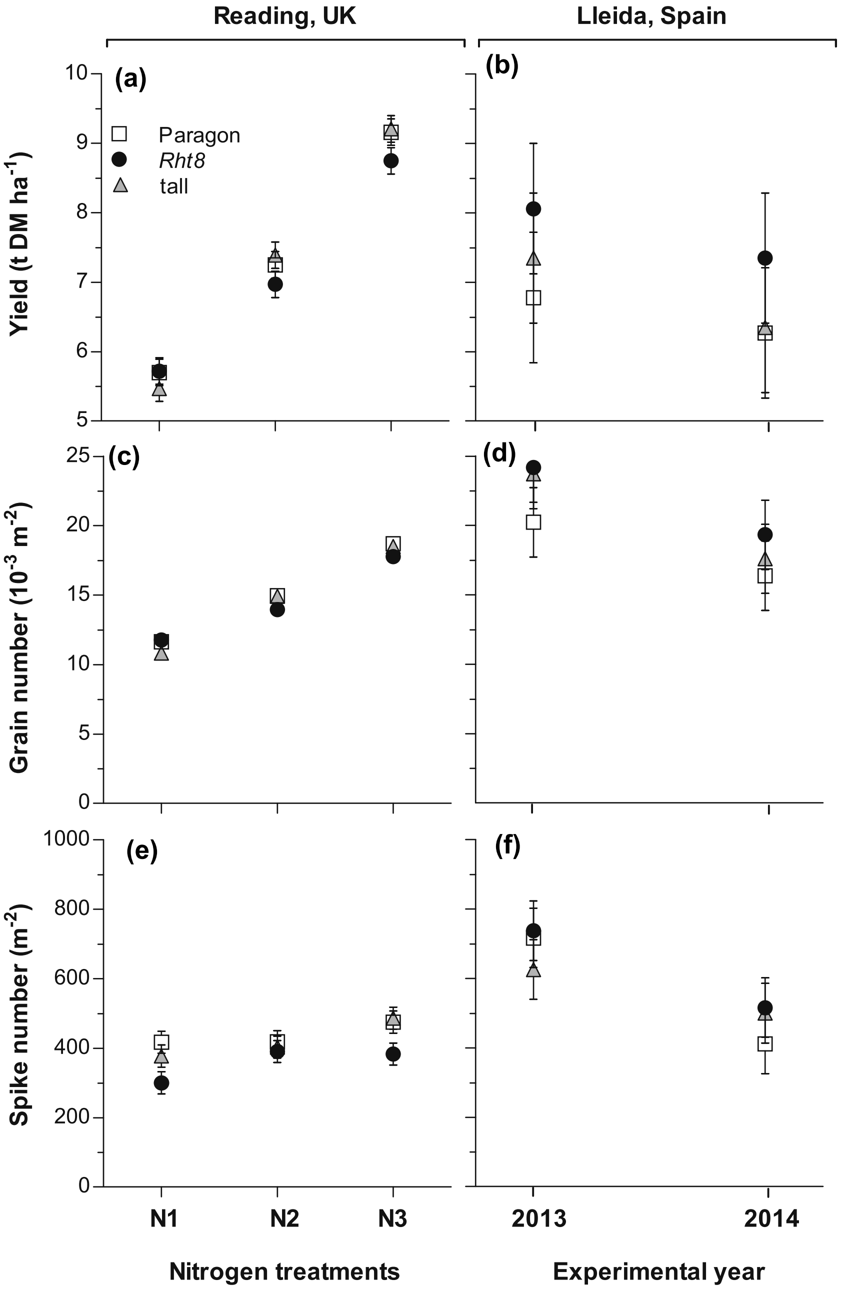

Fig. 4. Yield, grain number and spike number in the *Rht8* NIL, tall NIL and Paragon in Reading and Lleida. These data are the mean values ± SEM (standard error of the mean). Nitrogen treatment was N1 (40 kg N ha^{-1}), N2 (100 kg N ha^{-1}) and N3 (200 kg N ha^{-1}).

et al., 2014; Wang et al., 2015). A short and tall NIL contrasting for an introgression harbouring *Rht8* (short)/*rht8* (tall) were selected from a BC_3F_3 population of NILs in a UK spring wheat. The *Rht8* and tall NIL were grown alongside the recurrent parent across two sites in the UK and a high-temperature irrigated site in Spain. The high-temperature site was representative of southern Europe where *Rht8* is prevalent, since it provides adaptation to the hot summer conditions (Worland and Law, 1986) by providing a semi-dwarf phenotype with no effect on coleoptile length and early seedling vigour (Rebetzke and Richards, 2000; Ellis et al., 2004). We further investigated the effects of *Rht8* in low N-input systems and in irrigated conditions.

Plant height at maturity was reduced by ~11% in the *Rht8* NIL consistently across environments and different N treatments and water regimes. The reduction was principally due to the shortening of the spike, peduncle and second internode from the top. Although our study reports on the effects associated with *Rht8* in a single genetic background, the reduction in stature we report is within the 7–18% range associated with *Rht8* in different genetic backgrounds and environmental conditions (Ellis et al., 2004; Rebetzke et al., 2012; Wang et al., 2015). The stable height reduction across environments differs with the findings in a study of *Rht8* in a spring wheat background in Colorado, where the magnitude of height reduction tended to be smaller in lower yield-potential environments (Lanning et al., 2012). The differences in that study could reflect the reliance of the microsatellite marker *Xgwm261* as diagnostic for the presence of the *Rht8* allele, which is not always reliable (Ellis et al., 2007), or environmental differences. For example, the environments sampled by Lanning et al., 2012 varied

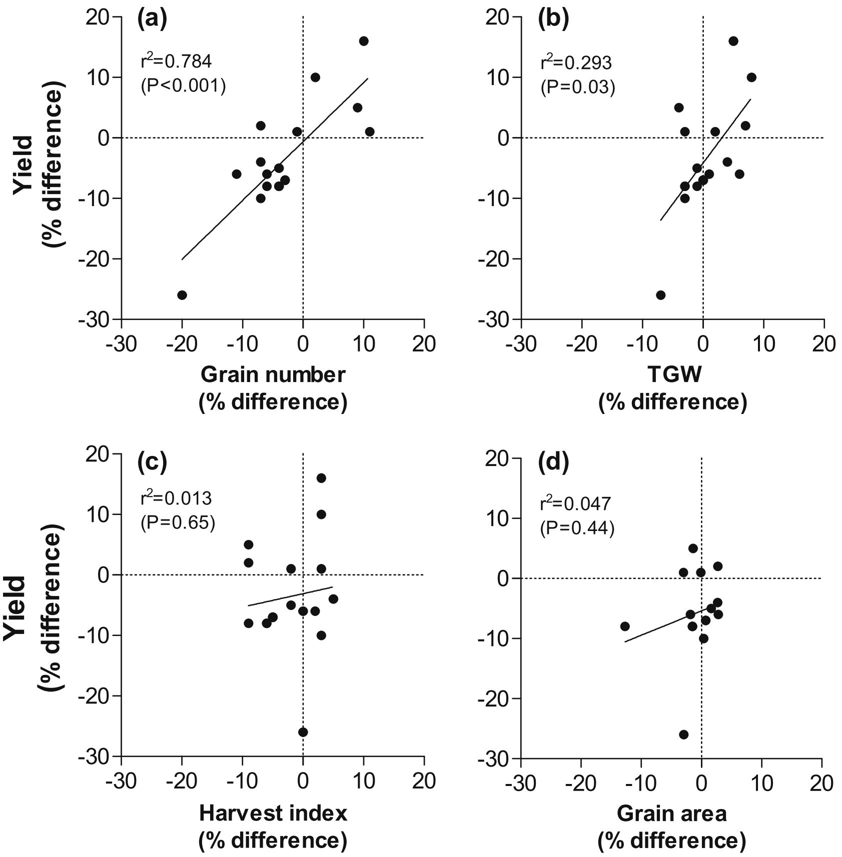

Fig. 5. Relationship between the yield and main yield components in the *Rht8* NIL as a percentage difference relative to the tall NIL for each environmental condition (experiment and treatment within experiment).

greatly for precipitation, whereas in our study the lowest-yielding environment was irrigated to field capacity.

Given the reduced stature of the *Rht8* NIL, caution was taken in experimental design to avoid shading by taller neighbouring plots. In Reading and Lleida, yield and yield components were measured from centrally-harvested plots. In Norwich, whole plots were combined however sufficient distance between plots minimised edge effects.

In high yield-potential UK environments under standard agronomic conditions of high N and no irrigation (rainfed), there was an average ~10% penalty associated with the *Rht8* introgression which is in line with previous reports of the yield disadvantage of *Rht8* in a different spring and winter-wheat *Ppd-D1a* background (Lanning et al., 2012; Wang et al., 2015). The key novel findings of our study are that the yield penalty in the *Rht8* NIL was overcome at low N, in irrigated conditions and at the high-temperature, lowest yield-potential site in Spain. This indicates that *Rht8* might be usefully deployed in low-input agriculture including, but not limited to, organic systems where yield stability is often more important than maximal yield (Wolfe et al., 2008). Further work using other NIL pairs from our BC_3F_3 population is needed to test and clarify this.

In the highest yield-potential environment, the yield penalty in the *Rht8* NIL under rainfed conditions was mitigated upon irrigation. At the high-temperature site, no yield penalty was associated with the *Rht8* introgression and instead the *Rht8* NIL had a mean 13% higher yield across both years compared to the tall NIL, though this was only significant at P = 0.07. Since the PAR interception of the *Rht8* NIL was lower than the tall NIL and the reduction was more severe as the growing season continued, it is possible that in the UK the yield of the *Rht8* NIL was compromised due to insufficient solar radiation (half the levels of Spain).

Within yield components, the strongest relationship between changes in yield in the *Rht8* NIL relative to tall was with grains m^{-2} and to a smaller extent grain weight. There were no significant relationships between yield response in the *Rht8* NIL and harvest index or grain area relative to the tall. There have been mixed findings of effects of *Rht8* on yield components, with significant differences reported in grain weight but not in grain number (Rebetzke et al., 2012; Wang et al., 2015). The reasons for these different findings could be due to environment and genetic background, for example some previous reports were in a winter-wheat *Ppd-D1a* background (Wang et al., 2015). Another reason is the different plot sizes used: the previous studies investigated traits at either the single plant level in pots or in small field plots comprising two rows, whereas larger plots were used in our study.

The concomitant reduction in spikes m^{-2} with reduction in yield associated with the *Rht8* introgression was a novel finding in our study. Unfortunately we did not measure anthesis date systematically in each location to determine whether the effect associated with the *Rht8* introgression on spike number would be independent of its possible effect on phenology. In the experiments where anthesis was determined (Norwich and Lleida), time to anthesis was not significantly affected in the *Rht8* NIL relative to the tall. Therefore it seems likely that the reduction in spikes m^{-2} in the *Rht8* NIL in Reading was genuine and independent of advancement in anthesis.

The yield component spikes m^{-2} is determined by tiller dynamics early on in development, although little is known about the molecular basis of tiller formation in wheat (Sreenivasulu and

Schnurbusch, 2012). A study of durum wheat in response to N and water availability found that increasing N early in development increased grains m^{-2} by increasing both the number of fertile florets and the proportion of florets setting grains (Ferrante et al., 2013). The interplay found between grains m^{-2}, spikes m^{-2} and yield in our study suggest that further investigation into tiller dynamics and floret generation, both established early in wheat development, is key to further explaining our findings. This is further strengthened by the reduced PAR interception in the *Rht8* NIL compared to tall which was more severe as the canopy matured. This indicates differences in the canopy and use of solar radiation associated with the *Rht8* introgression, which could be linked to tiller formation. Further, the findings raise the possibility that the yield reduction associated with *Rht8* in our study might be mitigated by increasing sowing rate and/or reducing row width.

We found that improved root-lodging resistance was associated with the *Rht8* NIL at agronomically-relevant N levels. However, since these were the same conditions which resulted in lower yield and reduced spike number, investigation into root characteristics known to reduce root-lodging risk (Berry et al., 2007) is required to dissect the mechanisms involved.

The anecdotally reported semi-compact spike phenotype associated with *Rht8* was quantified here for the first time. In our study, without the confounding effects of *Ppd-D1a*, a robust, significant increase in spike compaction of ~15% was observed in the *Rht8* NIL across environments and independent of N and irrigation treatments. In agreement with other studies (Sourdille et al., 2000; Jantasuriyarat et al., 2004; Faris et al., 2014), despite spike compaction being a function of spike length and spikelets per spike, the differences in compaction were driven only by changes in spike length.

The dominant allele of the gene *compactum* (*C*) defines a subspecies of hexaploid wheat known as club wheat, which is characterised by a spike half the length of common wheat and associated with lower grain weight and greater grain number per spike (Gul and Allan, 1972; Zwer et al., 1995). *C* was mapped to two bins either side of the 2D centromere (Johnson et al., 2008). A more recent publication stated that *C* was located on 2DS, based on personal communication (Faris et al., 2014). The *C* bin on 2DS maps 0.7cM proximal to the bin containing both markers used to introgress the *Rht8* segment in our population (*Xgwm261* and *Xcfd53*) (Johnson et al., 2008), though linkage drag might include the adjacent regions. Further, a spike compactness QTL on 2DS overlaps the *Xgwm261-Xcfd53* region (Manickavelu et al., 2011). It should be noted that neither of these previous studies used materials segregating for *Ppd-D1*, which is estimated be 10 cM distal to *Xcfd53* (Gasperini et al., 2012), in their genetic maps and *Ppd-D1* is known to influence spike morphology (Worland et al., 1998).

We observed consistent differences between the tall NIL and the recurrent parent to the population in plant height and spike compaction, which indicated background genetic effects. Genotyping using a high-density SNP array showed variation between the *Rht8* NIL and the recurrent parent outside the 2DS introgression was present, but smaller than the background expected in a third-generation back-cross. Taken together, finer genetic dissection of *Rht8* and *C* is required to determine the precise contribution of *Rht8*, *C* and linkage blocks around those loci (assuming they are distinct) to the agronomic traits presented in this study.

5. Conclusion

Investigation of the effects associated with the *Rht8* introgression in contrasting N treatments and water regimes indicated a robust height reduction in the *Rht8* NIL regardless of environment and treatment. The lengths of the spike, peduncle and second internode all contributed to the height reduction. A novel finding in our study was a reduction in spike number associated with *Rht8* at agronomically-relevant N levels, in the same conditions which resulted in yield reductions and reduced grain number. This is the first study to report that the yield penalty associated with *Rht8* was abolished at low N and in irrigated conditions; these benefits were replicated across sites and seasons. The *Rht8* introgression decreased spike length with constant spikelet number, resulting in a semi-compacted spike. Improved genetic resolution of *Rht8* and *compactum* is required to precisely dissect the contributions of these two loci on agronomic traits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fcr.2016.02.026.

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