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ORIGINAL ARTICLE

Identification and characterization of *Rht25*, a locus on chromosome arm 6AS affecting wheat plant height, heading time, and spike development

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Abstract

Key message **This study identified *Rht25*, a new plant height locus on wheat chromosome arm 6AS, and characterized its pleiotropic effects on important agronomic traits.**

Abstract Understanding genes regulating wheat plant height is important to optimize harvest index and maximize grain yield. In modern wheat varieties grown under high-input conditions, the gibberellin-insensitive semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* have been used extensively to confer lodging tolerance and improve harvest index. However, negative pleiotropic effects of these alleles (e.g., poor seedling emergence and reduced biomass) can cause yield losses in hot and dry environments. As part of current efforts to diversify the dwarfing alleles used in wheat breeding, we identified a quantitative trait locus (*QHt.ucw-6AS*) affecting plant height in the proximal region of chromosome arm 6AS (< 0.4 cM from the centromere). Using a large segregating population (~ 2800 gametes) and extensive progeny tests (70–93 plants per recombinant family), we mapped *QHt.ucw-6AS* as a Mendelian locus to a 0.2 cM interval (144.0–148.3 Mb, IWGSC Ref Seq v1.0) and show that it is different from *Rht18*. *QHt.ucw-6AS* is officially designated as *Rht25*, with *Rht25a* representing the height-increasing allele and *Rht25b* the dwarfing allele. The average dwarfing effect of *Rht25b* was found to be approximately half of the effect observed for *Rht-B1b* and *Rht-D1b*, and the effect is greater in the presence of the height-increasing *Rht-B1a* and *Rht-D1a* alleles than in the presence of the dwarfing alleles. *Rht25b* is gibberellin-sensitive and shows significant pleiotropic effects on coleoptile length, heading date, spike length, spikelet number, spikelet density, and grain weight. *Rht25* represents a new alternative dwarfing locus that should be evaluated for its potential to improve wheat yield in different environments.

Introduction

Wheat is one of the most widely grown crops in the world and provides approximately 20% of the calories in the human diet. Therefore, increasing wheat yields is essential to ensuring food security for a growing world population. One critical trait that facilitated the large yield gains achieved during

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the "Green Revolution" was reduced plant height (PH), as shorter stature reduces the incidence of lodging, particularly when plants are grown under high fertilizer regimes (Hedden 2003). In addition, shorter plants exhibit improved assimilate partitioning to developing spikes, resulting in a higher rate of floret survival, increased grain number per spike, and improved harvest index (Flintham et al. 1997; Youssefian et al. 1992).

The reduced PH of modern wheat cultivars was achieved through the introduction of semi-dominant dwarfing alleles at the *Reduced height* (*Rht*) loci on chromosome arms 4BS (*Rht-B1*) and 4DS (*Rht-D1*) (Peng et al. 1999). Both genes encode proteins designated DELLA, which are negative regulators of the gibberellin (GA) signaling pathway (Hauvermale et al. 2012). GAs comprise an important class of plant hormones with well-characterized roles in growth promotion during almost every stage of plant development (Hedden and Thomas 2012). Upon binding to a specific receptor, bioactive GAs initiate the formation of a protein complex which targets DELLA proteins for poly-ubiquitination and degradation by the 26S proteasome (Sun 2011). Although there are some DELLA-independent GA responses (Livne et al. 2015), the majority of characterized GA signaling processes are regulated primarily through GA-mediated degradation of DELLA proteins. The *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) semi-dwarfing alleles carry point mutations introducing premature stop codons in the N-terminal DELLA domain of their respective proteins. It has been suggested that translation reinitiates after the stop codon, producing a GA-insensitive, constitutively active repressor (Peng et al. 1999). Several other GA-insensitive *Rht1* alleles with similar effects also carry disruptive mutations within this domain (Pearce et al. 2011).

Although *Rht-B1b* and *Rht-D1b* contribute to increased grain yield under optimal environments, their benefits are less clear under hot and dry conditions. The adoption of these alleles in Southern Europe has been limited to regions not subjected to heat stress (Worland 1986), and no clear yield advantage of *Rht-B1b* and/or *Rht-D1b* was found in rainfed environments in Australia (Richards 1992) and west central Great Plains of North America (Butler et al. 2005). These alleles also reduce coleoptile length and early vigor, which can further penalize yield through poor seedling establishment (Rebetzke et al. 2007). This is a particular problem in environments with arid soils and limited precipitation such as inland Pacific Northwest (Schillinger et al. 1998) and Australia (Rebetzke et al. 2007), which require deep sowing to better access soil moisture. The negative pleiotropic effects of the widely deployed *Rht-B1b* and *Rht-D1b* have motivated the search for alternative dwarfing alleles.

So far, 24 *Rht* genes (*Rht1–Rht24*) have been named and catalogued in wheat (McIntosh et al. 2017, 2013). Several of these names correspond to previous synonyms for the *Rht-B1* and *Rht-D1* genes: *Rht1* = *Rht-B1b*, *Rht2* = *Rht-D1b*, *Rht3* = *Rht-B1c*, *Rht10* = *Rht-D1c*, *Rht11* = *Rht-B1e*, and *Rht17* = *Rht-B1p* (Börner et al. 1996; Divashuk et al. 2012; Bazhenov et al. 2015). The dwarfing alleles of these two genes are GA-insensitive, whereas for all the other named *Rht* genes both height-increasing and dwarfing alleles are responsive to GA (GA-sensitive) (McIntosh et al. 2017). Among the latter, *Rht8* on chromosome arm 2DS is one of the most extensively studied (Gasperini et al. 2012; Korzun et al. 1998) and was widely used in southern and eastern regions of Europe (Borojevic and Borojevic 2005). *Rht8b* has been shown to reduce final PH without affecting coleoptile length or seedling vigor (Rebetzke and Richards 2000; Rebetzke et al. 1999, 2007; Schillinger et al. 1998), and early studies suggested a positive effect on grain yield (Börner et al. 1993; Rebetzke and Richards 2000). However, more recent studies using near-isogenic lines have reported yield penalties of *Rht8b* in some environments, including high and low yielding sites in the northwestern USA (Lanning et al. 2012) and high yielding conditions in the UK (Kowalski et al. 2016).

As part of our efforts to identify additional genes regulating wheat PH, we precisely mapped *QHt.ucw-6AS*, a PH locus on the proximal region of chromosome arm 6AS. Comparison of *QHt.ucw-6AS* with previously mapped *Rht* loci on chromosome 6A showed that it was a different gene, designated here as *Rht25*. *Rht25* confers a stable effect on PH across multiple environments and has significant genetic interactions with the GA-insensitive genes *Rht-B1* and *Rht-D1*. We also show that *Rht25* is GA-sensitive and exhibits pleiotropic effects on coleoptile length, heading date, spike length, spikelet number, spikelet density, and grain weight. The potential value of *Rht25* for wheat breeding is discussed.

Materials and methods

Mapping populations

The initial QTL mapping was conducted using a population of 186 recombinant inbred lines (RILs) from the cross 'UC1110/PI610750' developed by Lowe et al. (2011). UC1110 (Chukar///Yding//Bluebird/Chanate) is a semi-dwarf hard spring wheat variety developed by the University of California, Davis. PI610750 [CIMMYT accession number CYG90.248.1, Croc1/*Aegilops tauschii* (Synthetic205)//Kauz] is a semi-dwarf synthetic derivative from the tetraploid (genomes AABB) variety Croc1 combined with the D genome progenitor of hexaploid wheat, *Aegilops tauschii* (genome DD). This variety was developed under the Wide Cross Program of the International Maize and Wheat Improvement Center (CIMMYT) in Mexico and was

originally registered for its resistance to *Septoria tritici* leaf blotch (Mujeeb-Kazi et al. 2000).

UC1110 carries the dwarfing allele *Rht-D1b*, and PI610750 carries the dwarfing allele *Rht-B1b*; so the resulting RIL population segregates for both PH genes. Analysis of PH data from this population revealed the presence of a third PH QTL on chromosome 6A, which was validated in this study. The UC1110/PI610750 RIL population was grown in Davis, CA, USA (38°32′N, 121°44′W), Tulelake, CA, USA (41°57′N, 121°28′W), and Marcos Juárez, Córdoba, Argentina (32°42′S, 62°07′W) during multiple years as described in Table 1.

To generate a more detailed map of the 6A QTL for PH and study its interactions with *Rht-B1* on coleoptile length and GA sensitivity, we generated a population of 64 F_2 plants from the cross between two selected UC1110/PI610750 RILs, 'RIL99/RIL106.' This population showed segregation for the 6A PH QTL and *Rht-B1* but was fixed for the height-increasing *Rht-D1a* allele. As detailed in "GA sensitivity assay" section, we selected plants segregating for both the 6A PH QTL and *Rht-B1* for the GA sensitivity experiments.

For the high-resolution map, we selected F_2 plants from the RIL99/RIL106 population that were fixed for the height-increasing *Rht-B1a* and *Rht-D1a* alleles but segregating for the 6A QTL region. The high-resolution map was developed in three recurrent rounds. In each round, plants segregating for the QTL region were screened for recombination events using molecular markers for loci flanking the candidate region. Progeny from plants carrying recombinant chromosomes (70–93 plants per family) was evaluated for PH to infer the genotype of the gene underlying the 6A QTL and to transform the QTL into a Mendelian locus. New markers were developed in each round to narrow the candidate gene region. The new flanking markers were used to identify plants carrying new recombination events and iterate the process described above in a narrower region.

To compare the chromosome location of the 6A QTL with *Rht18*, a previously known GA-sensitive PH gene on chromosome 6A, we developed a tetraploid F_2 mapping population ($n = 120$) segregating for *Rht18*. The parental lines for this population were the durum wheat variety 'Icaro' (PI503555) carrying the *Rht18* dwarfing allele in the *Rht-B1a* background and the durum wheat variety 'Langdon,' which carries *Rht-B1a* but lacks the *Rht18* dwarfing allele.

Genotyping and genetic map construction

The 3157 cM genetic map for the UC1110/PI610750 RIL population was previously described in Lowe et al. (2011). The map includes 229 polymorphic simple sequence repeat (SSR) and 1229 diversity arrays technology (DArT) markers,

Table 1 Description of experiments for the UC1110/PI610750 RIL population

Experiment	Location and year[a]	No. of blocks	Experimental unit	Traits phenotyped
I	DVS 2007	1	One meter row (30 seeds per row)	PH, HD
II	DVS 2008	2	One meter row (30 seeds per row)	PH, HD
III	TLL 2008	1	One meter row (30 seeds per row)	PH
IV	MsJz 2010	1	Pot (3 seeds per pot)	PH, HD, SL, StN, SD, GW
V	MsJz 2010	1	Hill plot (5 seeds per plot)	PH, SL, StN, GW
VI[b]	MsJz 2011	2	Hill plot (5 seeds per plot)	PH, HD, SL, StN, SD, GW
VII[b]	MsJz 2011	2	Hill plot (5 seeds per plot)	PH, HD, SL, StN, SD, GW
VIII	MsJz 2012	2	Hill plot (5 seeds per plot)	PH, HD, GW, GN
IX	MsJz 2012	1	One meter row (30 seeds per row)	PH, HD, GW, GN
X	MsJz 2013	2	One meter row (30 seeds per row)	PH, HD

PH plant height, *HD* heading date, *SL* spike length, *StN* spikelet number per spike, *SD* spikelet density, *GW* grain weight, *GN* grain number per spike

[a]All experiments were performed in the field except for experiment IV (MsJz 2010), which was conducted in a greenhouse. DVS: Davis, CA, USA, TLL: Tulelake, CA, USA, MsJz: Marcos Juárez, Córdoba, Argentina. Experiments in MsJz were rain fed and those in DVS and TLL were irrigated

[b]Experiments VI and VII differ in planting date (June 1st and June 20th, respectively)

underlying 559 unique loci distributed across the 21 wheat chromosomes. The following four additional markers were added to the RIL map for this QTL study: *Rht-B1* and *Rht-D1* sequence-tagged site (STS) markers (Ellis et al. 2002), *wmg4603* SSR marker (TraitGenetics, Gatersleben, Germany), and *IWA3866* SNP marker (Cavanagh et al. 2013) on chromosome 6A. For the high-density maps, we added 12 SNPs selected from the wheat 90K SNP array (Wang et al. 2014) and 18 SNPs identified by exome-sequencing RIL143 (6A QTL dwarfing allele) and PI610750 (6A QTL height-increasing allele) (Table S1). Exome capture was performed using Roche SeqCap EZ probes (140430_Wheat_TGAC_D14_REZ_HX1 for T. *aestivum*) as described before (Krasileva et al. 2017). Exome library sequencing and sequence data analysis were conducted as previously described by Mo et al. (2018) to produce the binary alignment/map (BAM) files of the two parents mapped to the IWGSC RefSeq v1.0 reference. Variant calling was conducted using 'SAMtools' (Li et al. 2009) (version 0.1.19; command "mpileup" with default parameters) and 'BCFtools' (Li 2011) (version 0.1.19; command "call" with default parameters except-m).

The Icaro/Langdon F_2 population was genotyped using two SSRs (*barc3* and *wmg4603*) and 11 SNPs from chromosome 6A. Eight of the 11 SNPs were identified in the current study by exome-sequencing Icaro and Langdon (Supplementary Table S1), using the same SeqCap EZ probes and sequence data analysis described above. All genetic linkage maps were constructed with MAPMAKER/EXP 3.0 (Lander et al. 1987) using the Kosambi distance function (Kosambi 1943).

SNP genotyping was conducted using KASP (Kompetitive Allele Specific PCR, LGC-Genomics, UK) assay with primers designed using PolyMarker (Ramirez-Gonzalez et al. 2015). Genomic coordinates for molecular markers and genes in this study refer to the Chinese Spring IWGSC RefSeq v1.0 (https://urgi.versailles.inra.fr/blast_iwgsc/blast.php). SNP markers developed from the exome capture were designated with the chromosome name followed by the first five numbers of the pseudomolecule coordinates based on RefSeq v1.0 (e.g., *6A11532* for a SNP located at 115.32 Mb on chromosome 6A). The complete pseudomolecule coordinates for each of the mapped SNPs are provided in Supplementary Table S1.

Phenotyping

The experiments for the initial QTL mapping with the RIL population included ten trials described in Table 1 (referred to as experiments I–X). All experiments for QTL mapping were performed in the field except for experiment IV that was performed in a greenhouse. In experiments II, VI, VII, VIII, and X, all lines were replicated in two blocks in a randomized complete block design (RCBD). In the rest of the experiments, each line for the complete population was included only once (Table 1).

The RIL population was evaluated for seven agronomic traits: heading date, PH, spike length, spikelet number per spike, spikelet density, grain number per spike, and grain weight. Heading date (experiments I, II, IV, and VI–X) was measured in days from emergence until 50% of the spike had emerged from the flag leaf in 50% of the plants (Zadoks et al. 1974). Plant height (experiments I–X) was determined after maturity as the average of ten randomly selected plants by measuring the tallest tiller of each plant in centimeters from the ground to the top of the spike excluding awns. Spike length (experiments IV–VII) was determined at the end of the life cycle as the average of ten randomly selected spikes measured in centimeters from the base to the top of the spike excluding awns. Spikelet number per spike (experiments IV–VII) was determined at the end of the life cycle as the average number of spikelets in ten randomly selected spikes. Spikelet density was calculated by dividing the average spikelet number per spike with the average spike length. Grain number per spike (experiments VIII and IX) was determined after harvest as the average of the total number of grains in ten randomly selected spikes. Grain weight (experiments IV–IX) was determined after harvest by weighting 1000 grains and calculating the average grain weight in milligrams.

Progeny tests for high-resolution mapping of the 6A PH QTL were conducted in Davis, CA, USA. Progeny tests for the first and the second mapping rounds were conducted in the field in 2015 and 2016, respectively. Plants were grown in 1 m rows, with five plants per row. Progeny tests for the third mapping round was conducted in a greenhouse in 2017, in which each plant was grown in a cone-shaped pot with 6.9 cm diameter and 25.4 cm depth. Plant height was determined after maturity by measuring the tallest tiller of each plant.

The Icaro/Langdon F_2 population was grown in the field in Tulelake, CA, USA in 2017. Plants were grown in 1 m rows, with five plants per row. Plant height was determined as described above.

QTL and statistical analyses

QTL analysis for the UC1110/PI610750 RIL population and the Icaro/Langdon F_2 population were conducted using composite interval mapping (CIM) with forward and backward regressions and 500 permutations at $\alpha = 0.05$ as implemented in the publicly available software QTL Cartographer 2.5 (Wang et al. 2012). For the UC1110/PI610750 RIL population, the QTL analysis was performed on data for each environment separately, after averaging the scores for each RIL across all replications within each trial. A LOD value of 3.0 was selected as a uniform threshold for all analyses.

For each of the traits evaluated in the RIL population, we performed a factorial ANOVA using *Rht-B1*, *Rht-D1,* and the marker at the peak of the 6A PH QTL as class variables in the model, together with all possible two- and three-way interactions. Trials (experiments) were included as blocks (a random class variable). This analysis was used to determine the potential epistatic interactions among loci and to estimate variance components using the restricted maximum likelihood (REML) model (Corbeil and Searle 1976). For the progeny tests, one-way ANOVAs were conducted separately for each family using a marker within the segregating chromosome region in that family as a class variable. Data violating the ANOVA assumptions (normality of residuals by Shapiro–Wilk and Kolmogorov–Smirnov tests, and homogeneity of variances by Levene's tests) were corrected using power transformations. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).

GA sensitivity assay

GA sensitivity assays were conducted using RIL99/RIL106 derived F_4 seeds. We first selected a plant from the RIL99/RIL106 F_2 population (see "Mapping populations" section) that was heterozygous for both *Rht-B1* and the 6A PH QTL using the *Rht-B1* STS marker (Ellis et al. 2002) and the peak marker for the 6A PH QTL. Among the $F_{2:3}$ progeny, we selected plants homozygous for each of the four possible combinations of the two segregating PH loci and used their F_4 seeds to evaluate coleoptile length under different GA concentrations.

F_4 seeds were sown 2.5 cm below the top edge of germination paper (26 cm × 13 cm) moistened with distilled water. The germination paper was rolled and kept at 4 °C for 48 h, and moved to GA_3 (Sigma-Aldrich, St. Louis, MO, USA) solutions with different concentrations (0, 0.1, 1.0, and 10 µM; dissolved in distilled water) at room temperature. The rolled germination paper with seeds was kept upright, with the bottom 4 cm of the paper soaked in the assigned GA solution. After 10 days, coleoptile lengths were measured. The experiments were conducted as an RCBD with four blocks and one replication per block-treatment combination. Each replication was represented by the average coleoptile lengths from eight plants (subsamples). The experiment was replicated three times using F_4 seeds from different F_3 sister lines.

The data were analyzed using two-way ANOVA with *Rht-B1* and the marker at the peak of the 6A QTL as class variables and the three replications with different sister lines as blocks, using SAS 9.4. The GA sensitivity for each genotype was determined using a GA response index (GRI), calculated as the percent increase in coleoptile length under the highest GA concentration (10 µM) relative to the control (0 µM).

Results

QTL mapping for PH with the UC1110/PI610750 RIL population

Mean RIL PH values from the ten environments showed transgressive segregation and a bimodal distribution. A majority (93%) of plants carrying both *Rht-B1b* and *Rht-D1b* formed a group of very short plants (43–59 cm), whereas the RILs with all other allele combinations showed an approximate normal distribution (Supplementary Fig. S1a, Shapiro–Wilk $P = 0.5781$). A QTL analysis of the UC1110/PI610750 RIL population for PH identified three major effects on chromosome arms 4DS, 4BS, and 6AS, which were consistent across all ten environments (Fig. 1). To visualize the relative contribution of these three loci, we plotted the difference in plant height between the two alleles of each gene (Supplementary Fig. S2a) and the percentage of variance explained by each gene in a factorial ANOVA (Supplementary Fig. S2b) across the ten environments. These graphs showed that the magnitude of the effects of these genes is affected by the environment, but that their relative contributions are relatively stable across environments.

The 4DS QTL showed the largest effect, and the allele for short PH was contributed by UC1110. The peak of this QTL was mapped at the *Rht-D1* gene STS marker (43.7 cM, Fig. 1a). This result is consistent with the presence of the dwarfing *Rht-D1b* allele in UC1110 and the height-increasing *Rht-D1a* allele in PI610750. In the factorial ANOVA, *Rht-D1* explained 31.8% of the observed variation in PH (Table 2). The average difference between plants homozygous for *Rht-D1a* and *Rht-D1b* was 21.0 cm.

The 4BS QTL showed the second largest effect on PH, and the dwarfing allele was contributed by PI610750. The peak of this QTL was mapped at the *Rht-B1* gene STS marker (35.3 cM, Fig. 1b). This result is consistent with the presence of *Rht-B1b* in PI610750 and *Rht-B1a* in UC1110. In the factorial ANOVA, *Rht-B1* accounted for 20.0% of the observed variation in PH (Table 2). The average difference between plants homozygous for *Rht-B1a* and *Rht-B1b* was 16.8 cm.

A third PH QTL in the proximal region of chromosome arm 6AS (henceforth *QHt.ucw-6AS*) was significant in the nine field experiments (LOD > 3.0). In the greenhouse experiment (Table 1, experiment IV), its LOD score was below the selected threshold (LOD = 2.5, Fig. 1c). The peak of *QHt.ucw-6AS* was mapped at the *barc3* locus (110.7 cM; IWGSC RefSeq v1.0 6A pseudomolecule 85.3 Mb), flanked by markers *wmc754-6A* (102.1 cM; 52.8 Mb) and *wmg4603* (113.9 cM; 260.5 Mb, Fig. 1c). The proximal SSR marker *wmg4603* is located on the short

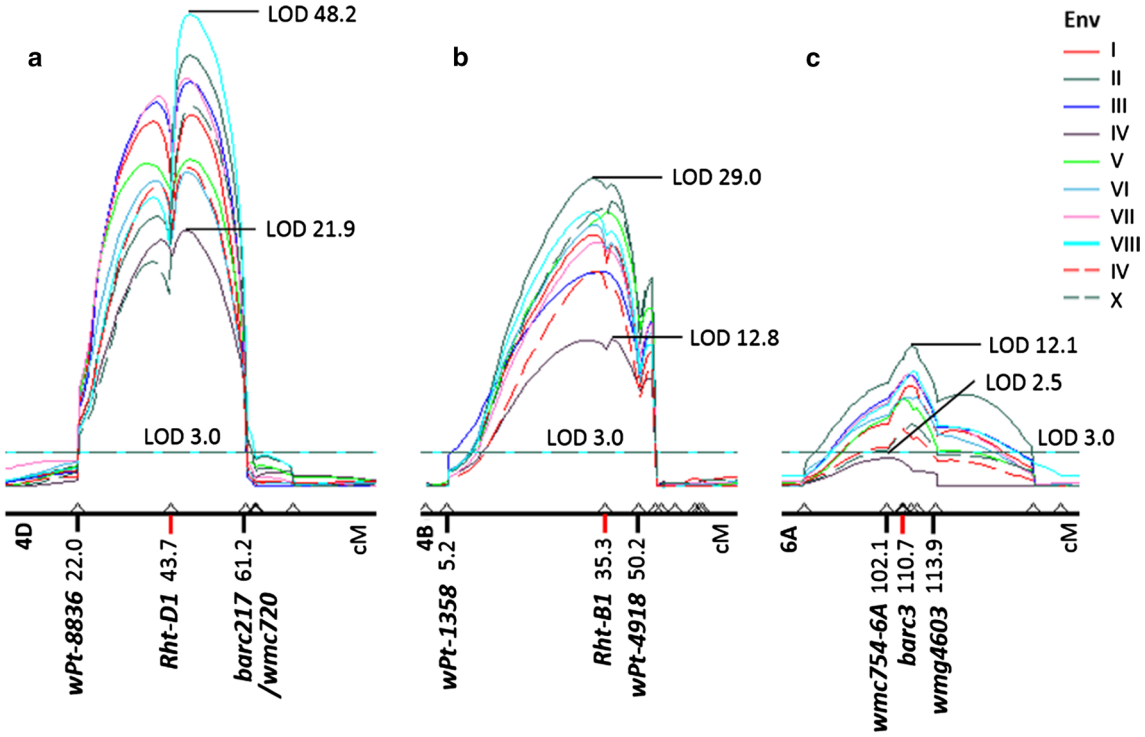

Fig. 1 QTLs for PH identified from the UC1110/PI610750 RIL population on chromosomes **a** 4D, **b** 4B, and **c** 6A. The highest and the lowest peak LOD scores among the 10 environments are indicated in each plot, along with the horizontal line indicating the threshold LOD (3.0). The three plots are in the same scale to facilitate comparisons among genes. Lines of different colors indicate different environments (I–X; Table 1). Names of the peak markers (red tick marks) and their flanking markers are listed below their positions (cM)

arm, which indicates that *QHt.ucw-6AS* is located on chromosome arm 6AS. The *barc3* locus has been assigned to the Chinese Spring deletion bin 6AS1-0.35-1.00 (Somers et al. 2004), suggesting that *QHt.ucw-6AS* is not located in the most centromeric bin of this arm. Although *wmg4603* is only 3.2 cM proximal to *barc3*, these two markers are far apart (~ 175 Mb) in the 6A pseudomolecule.

The cultivated parent UC1110 contributed the dwarfing allele (henceforth *QHt.ucw-6ASb*) and the synthetic derived parent PI610750 the height-increasing allele (henceforth *QHt.ucw-6ASa*). Allele designations were selected to be consistent with the *Rht-B1* and *Rht-D1* allele designations (a = tall and b = short). In the factorial ANOVA, *QHt.ucw-6AS* explained 5.4% of the variation in PH (Table 2). The average difference between plants homozygous for *QHt.ucw-6ASa* and *QHt.ucw-6ASb* was 9.1 cm.

QHt.ucw-6AS exhibited significant ($P < 0.0001$) two-way interactions with *Rht-B1* and *Rht-D1* (Table 2, Fig. 2). Even though *QHt.ucw-6ASb* was associated with significantly reduced PH when combined with either allele of *Rht-B1* and *Rht-D1*, its dwarfing effect was greater in the presence of the height-increasing alleles than in the presence of the dwarfing alleles (Fig. 2a, b). A significant ($P = 0.0001$) interaction was also detected between *Rht-B1* and *Rht-D1* (Table 2). Unlike the interaction with *QHt.ucw-6AS*, the dwarfing effects of *Rht-B1b* and *Rht-D1b* were greater in the presence of the dwarfing allele for the other gene (Fig. 2c).

Effects of *Rht-B1*, *Rht-D1*, and *QHt.ucw-6AS* on other agronomic traits

We also analyzed the effects of *Rht-B1*, *Rht-D1*, and *QHt.ucw-6AS* on heading date, spikelet number per spike, spike length, spikelet density, grain number per spike, and grain weight. The distribution of RIL mean values across environments for each trait is illustrated in Supplementary Fig. S1b–g. The main effects of the three loci and their two-way interactions on each trait are summarized in Table 2.

Heading date

All three loci had highly significant effects on heading date ($P < 0.0001$; Table 2). Plants carrying the *Rht-B1b* and *Rht-D1b* dwarfing alleles headed 1.2 and 1.6 days later than plants carrying the height-increasing alleles, respectively. By contrast, plants carrying the *QHt.ucw-6ASb* dwarfing allele headed 1.6 days earlier than those carrying *QHt.ucw-6ASa* (Table 2). A significant ($P < 0.0001$) two-way interaction was detected between *Rht-B1* and *Rht-D1*, in which the effect of each gene on heading date was significant only in

Table 2 Effects of *Rht-B1*, *Rht-D1*, and *QHt.ucw-6AS* (*Q6AS*) on different agronomic traits

Trait	Value	Main effect			Two-way interaction		
		Rht-B1 (*B*)	*Rht-D1* (*D*)	*Q6AS* (*A*)	*B*×*D*	*B*×*A*	*D*×*A*
Plant height (cm)	Allele a (tall)	82.3	84.4	78.4			
N = 2415[a]	Allele b (short)	65.5	63.4	69.3			
Env = 10[b]	*P*	****	****	****	****	****	**
	Variation (%)	20.0	31.8	5.4	0.5	0.8	0.5
Heading date (days)	Allele a (tall)	108.0	107.8	109.4			
N = 2240	Allele b (short)	109.2	109.4	107.8			
Env = 8	*P*	****	****	****	****	*	ns
	Variation (%)	0.0	0.2	0.2	0.2	0.1	–
Spikelet number	Allele a (tall)	19.4	19.3	19.1			
N = 1030	Allele b (short)	19.6	19.7	19.9			
Env = 4	*P*	ns	**	****	ns	ns	ns
	Variation (%)	–	0.5	3.5	–	–	–
Spike length (cm)	Allele a (tall)	10.7	10.7	10.9			
N = 1030	Allele b (short)	10.8	10.7	10.6			
Env = 4	*P*	ns	ns	**	ns	ns	ns
	Variation (%)	–	–	0.6	–	–	–
Spikelet density (spikelet no./cm)	Allele a (tall)	1.84	1.83	1.78			
N = 1030	Allele b (short)	1.85	1.86	1.91			
Env = 4	*P*	ns	*	****	ns	ns	ns
	Variation (%)	–	0.1	1.4	–	–	–
Grain number per spike	Allele a (tall)	35.6	37.6	35.2			
N = 519	Allele b (short)	33.9	32.0	34.4			
Env = 2[c]	*P*	ns	****	ns	**	ns	ns
	Variation (%)	0.0	14.2	–	4.0	–	–
Grain weight (mg)	Allele a (tall)	36.4	37.2	36.1			
N = 1347	Allele b (short)	33.1	32.2	33.3			
Env = 6	*P*	****	****	****	*	ns	ns
	Variation (%)	3.4	8.0	2.6	0.1	–	–

UC1110 carries *Rht-B1a*, *Rht-D1b*, and *QHt.ucw-6ASb* while PI610750 carries *Rht-B1b*, *Rht-D1a*, and *QHt.ucw-6ASa*

[a]Number of experimental units measured. Number of blocks per environment is listed in Table 1

[b]Number of environments where the trait was evaluated

[c]For grain number the two environments were without irrigation

P-values are from three-way mixed-model ANOVAs with environment as a random variable and the three loci as fixed variables (*$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$, *ns* not significant). % variation explained was calculated using the REML method in SAS 9.4 and is shown only for significant effects

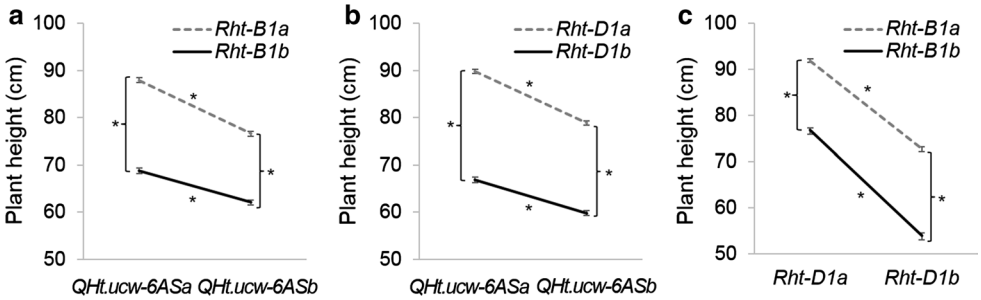

Fig. 2 Two-way interaction plots on PH between **a** *Rht-B1* and *QHt.ucw-6AS*, **b** *Rht-D1* and *QHt.ucw-6AS*, and **c** *Rht-B1* and *Rht-D1*. An asterisk indicates a significant ($P<0.05$) simple effect of each gene in the presence of each allele of the other gene, by Tukey's test. Error bars indicate ± 1 standard error

the presence of the dwarfing allele of the other gene (Supplementary Fig. S3a). The interaction between *Rht-B1* and *QHt.ucw-6AS* was marginally significant ($P = 0.0358$). The effect of *QHt.ucw-6AS* was significant only in the presence of *Rht-B1a*, and the effect of *Rht-B1* was significant only in the presence of *QHt.ucw-6ASb* (Supplementary Fig. S3b).

Spikelet number per spike

Rht-D1 ($P = 0.0054$) and *QHt.ucw-6AS* ($P < 0.0001$) both had significant effects on spikelet number, while the effect of *Rht-B1* was not significant. Plants carrying the dwarfing alleles for *Rht-D1* and *QHt.ucw-6AS* had 0.4 and 0.8 more spikelets per spike than those carrying the height-increasing alleles, respectively (Table 2). No significant interaction was observed between these two loci.

Spike length

QHt.ucw-6AS had a significant ($P = 0.0042$) effect on spike length, while the effects of *Rht-B1* and *Rht-D1* were not significant. Plants carrying *QHt.ucw-6ASa* had 0.3 cm longer spikes than those carrying *QHt.ucw-6ASb* (Table 2). No significant two-way interaction was observed.

Spikelet density

QHt.ucw-6AS showed a highly significant ($P < 0.0001$) effect on spikelet density, with *QHt.ucw-6ASb* associated with denser spikes (Table 2). The effect of *Rht-B1* was not significant and that of *Rht-D1* was marginally significant ($P = 0.0115$). No significant two-way interaction was observed.

Grain number per spike

Rht-D1 exhibited highly significant ($P < 0.0001$) effect on grain number, with *Rht-D1b* associated with fewer grains (Table 2). A significant ($P = 0.0027$) interaction was observed between *Rht-B1* and *Rht-D1*, in which *Rht-B1b* was significantly associated with fewer grains only under the presence of *Rht-D1b* (Supplementary Fig. S3c). Although the effects were not significant, plants carrying *QHt.ucw-6ASb* showed fewer grains per spike than plants carrying *QHt.ucw-6ASa*, a similar trend to the one observed for *Rht-B1* and *Rht-D1*. Grain number was evaluated only in two rainfed environments (Table 1). Therefore, the reduced number of grains associated with the dwarfing alleles in these locations may reflect the tendency of taller wheat plants to perform better in water-stressed environments (Butler et al. 2005; Richards 1992; Zhang et al. 2018).

Grain weight

All three loci had highly significant ($P < 0.0001$) effects on grain weight, in which the height-increasing alleles were associated with heavier grains. The strongest effect was observed for *Rht-D1* (4.9 mg), followed by *Rht-B1* (3.3 mg), and then *QHt.ucw-6AS* (2.8 mg) (Table 2). A marginally significant ($P = 0.0414$) interaction was detected between *Rht-B1* and *Rht-D1*, where the effect of each gene on grain weight was greater in the presence of the dwarfing allele of the other gene (Supplementary Fig. S3d).

Effect of *QHt.ucw-6AS* on GA sensitivity and coleoptile length

To quantify the GA sensitivity of the *QHt.ucw-6AS* and *Rht-B1* alleles, we used F_4 seeds harvested from the F_3 lines homozygous for each of the four possible allelic combinations of these two genes derived from the RIL99/RIL106 F_2 population (*Rht-B1a/QHt.ucw-6ASa*, *Rht-B1a/QHt.ucw-6ASb*, *Rht-B1b/QHt.ucw-6ASa*, and *Rht-B1b/QHt.ucw-6ASb*, see "Materials and methods" section, Fig. 3).

In the absence of exogenous GA, both *Rht-B1* ($P < 0.0001$) and *QHt.ucw-6AS* ($P = 0.0060$) showed a significant effect on coleoptile length (Fig. 3a). The dwarfing alleles were associated with reduced coleoptile length for both *Rht-B1b* (17.1%, 0.75 cm) and *QHt.ucw-6ASb* (4.5%, 0.18 cm). No significant interaction was detected between *Rht-B1* and *QHt.ucw-6AS* for coleoptile length.

While the tall genotype (*QHt.ucw-6ASa/Rht-B1a*) exhibited a significant increase in coleoptile length relative to the control under all tested GA concentrations (0.1, 1, and 10 µM; Dunnett's test $P < 0.05$), plants carrying either *Rht-B1b* or *QHt.ucw-6ASb* showed a significant increase in coleoptile length only under the highest GA concentration (10 µM; Dunnett's test $P < 0.05$; Fig. 3b).

The GA sensitivity for each genotype was determined using GRI as described in "Materials and methods" section, (Fig. 3c). The effect of *Rht-B1* on GRI was highly significant ($P = 0.0021$), with *Rht-B1b* plants showing significantly lower GRI compared to *Rht-B1a* plants. In contrast, the effect of *QHt.ucw-6AS* on GRI was not significant ($P = 0.3983$), indicating that both *QHt.ucw-6AS* alleles respond similarly to GA. Therefore, the dwarfing allele underlying *QHt.ucw-6AS* should be considered GA-sensitive. No significant ($P = 0.5827$) interaction on GRI was observed between *Rht-B1* and *QHt.ucw-6AS*.

High-density map of *QHt.ucw-6AS*

To map *QHt.ucw-6AS* as a simple Mendelian gene with high resolution, we performed three rounds of increasingly precise mapping including large progeny tests of the plants

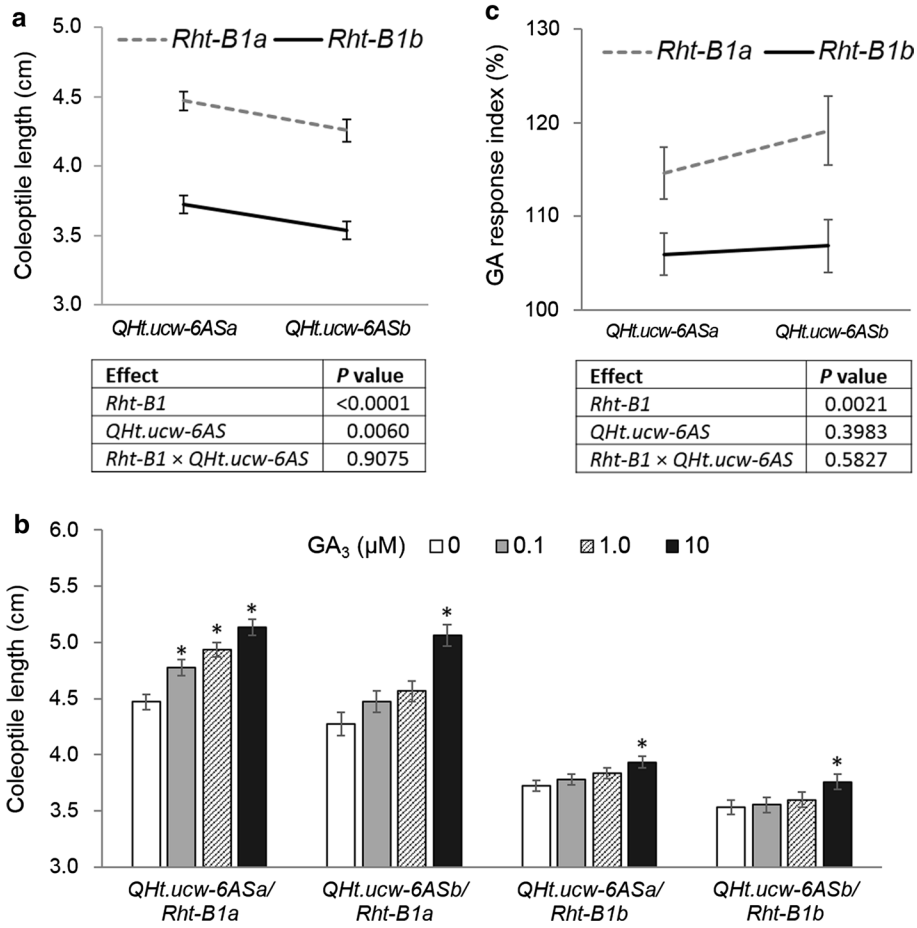

Fig. 3 Effects of *QHt.ucw-6AS* and *Rht-B1* on coleoptile length and GA sensitivity. **a** Two-way interaction between *QHt.ucw-6AS* and *Rht-B1* on coleoptile length in the absence of exogenous GA. **b** Coleoptile lengths of the four genotype combinations under different GA concentrations. An asterisk indicates a significant ($P < 0.05$) difference in comparison with the control (GA_3 0 μM) by Dunnett's test. **c** Two-way interaction between *QHt.ucw-6AS* and *Rht-B1* on GA response index [100 × (coleoptile length at GA_3 10 μM)/(coleoptile length at GA_3 0 μM)]. The reported *P*-values in panels **a**, **c** are from two-way ANOVAs. Error bars indicate ± 1 standard error

showing recombination events in the critical gene region (Table 3).

In the first round of mapping, 18 plants with recombination events between markers *wmc754* and *wmg4603* flanking *QHt.ucw-6AS* were identified by screening 224 F_3 plants derived from the RIL99/RIL106 F_2 homozygous for both *Rht-B1a* and *Rht-D1a* height-increasing alleles (Table 3). Plants carrying recombination events were genotyped with *wmc754*, *barc3*, *wmg4603*, and eight new SNPs developed in this region (Fig. 4a). Based on the distribution of recombination events, we selected seven $F_{3:4}$ families and performed large progeny tests including 82–93 plants per family (Supplementary Table S2). Plant height and genotype (based on one segregating marker) were determined for each plant and one-way ANOVAs were performed for each family. Two of the seven families showed significant segregation for PH associated with the segregating marker, while five families did not show significant differences in PH (Supplementary Table S2). Based on these results, *QHt.ucw-6AS* was mapped within a 3.4 cM region flanked by markers *IWB65184*/*barc3* and *IWA7431* (Fig. 4a). Of the four markers that did not recombine with *QHt.ucw-6AS* in 167 segregating plants, three (*IWB4536*, *IWB41674*, *wmg4603*) were located on the short arm and one (*IWA7323*) was located on the long arm of chromosome 6A (Fig. 4a; Supplementary Table S2).

In the second round of mapping, 16 plants carrying recombination events between *IWB65184*/*barc3* and *IWA7431* were identified by screening 185 F_4 plants

Table 3 Description of high-resolution mapping rounds for *QHt.ucw-6AS*

Mapping round	No. of plants screened	No. of recombinants identified	No. of progeny tests	No. of new markers	No. of plants per progeny	Environment (year)
I	224 (F_3)	18	7 ($F_{3:4}$)	8	82–93	Field (2015)
II	185 (F_4)	16	16 ($F_{4:5}$)	20	76–93	Field (2016)
III	1007 (F_5)	13	8 ($F_{5:6}$)	2	70–72	Greenhouse (2017)

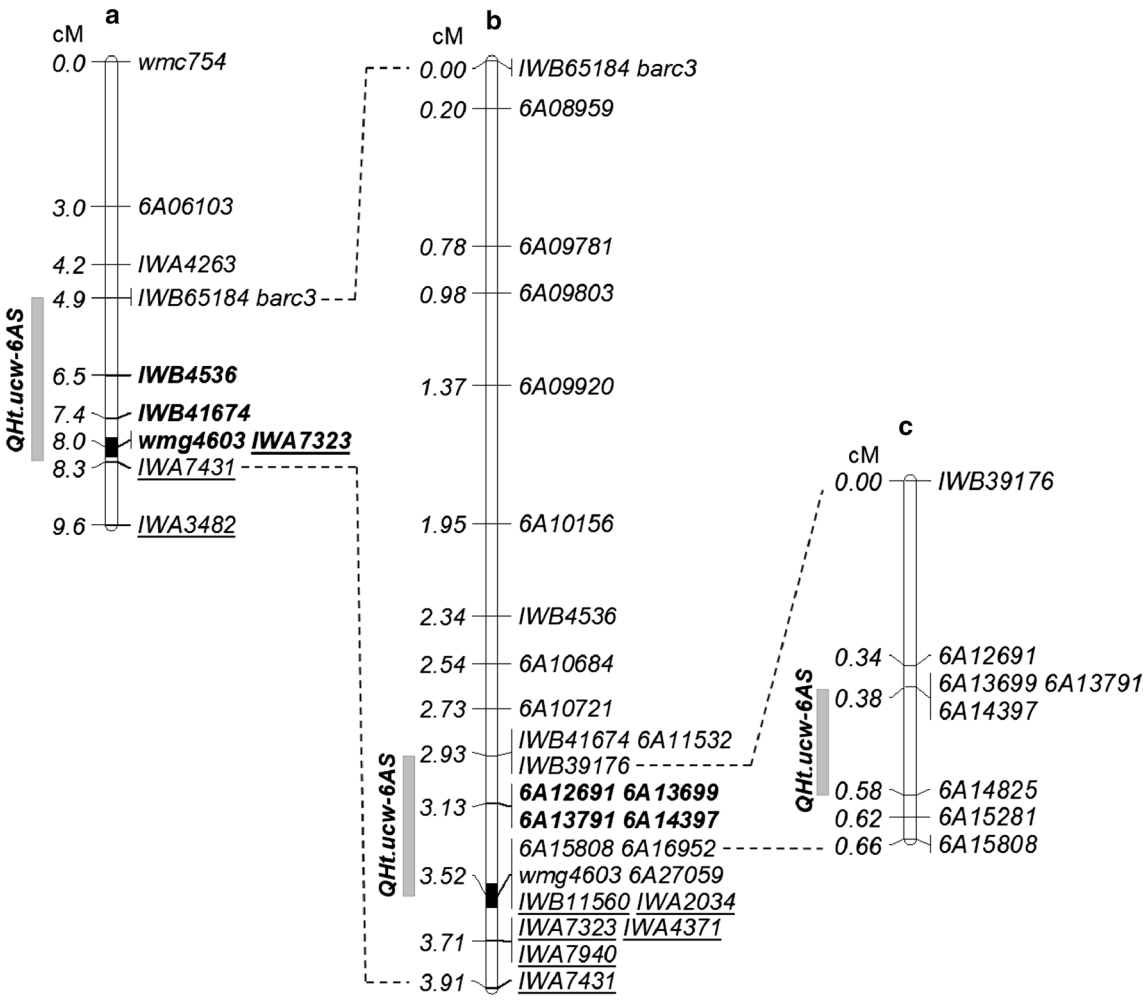

Fig. 4 Linkage maps of the *QHt.ucw-6AS* region on chromosome 6A. **a** Round I map from the $F_{3:4}$ progeny tests (Supplementary Table S2). **b** Round II map from the $F_{4:5}$ progeny tests (Supplementary Table S3). **c** Round III map from the $F_{5:6}$ progeny tests (Supplementary Table S4). Markers in bold did not recombine with *QHt.ucw-6AS* in 167 (**a**) and 256 (**b**) segregating plants. Markers located on chromosome arm 6AL are underlined, while those on chromosome arm 6AS are not underlined. Centromere positions are depicted as black boxes

derived from selected heterozygous F_3 plants (Table 3). Twenty additional markers were used to characterize the new recombination events (Fig. 4b). Progeny tests were performed for the 16 $F_{4:5}$ families using 76–93 plants per family. Plant height and genotype were determined for all plants and ANOVAs were performed for each family. Based on these results (Supplementary Table S3), *QHt.ucw-6AS* was mapped within a 0.59 cM region flanked by linked markers *IWB41674/6A11532/IWB39176* in the proximal side and *6A15808/6A16952/wmg4603/6A27059/IWB11560/IWA2034* in the distal side (Fig. 4b). Four markers (*6A12691*, *6A13699*, *6A13791*, and *6A14397*) did not recombine with *QHt.ucw-6AS* in 256 segregating plants and were located 0.39 cM from the centromere.

In the final round, 13 plants carrying recombination events between *IWB39176* and *6A15808* were identified by screening 1007 F_5 plants derived from heterozygous F_4 plants (Table 3). Two additional SNPs were mapped in the region and progeny tests were conducted using eight $F_{5:6}$ families (70–72 plants per family). Genotypes and PH were determined for all plants (Supplementary Table S4). Based on the ANOVAs, *QHt.ucw-6AS* was mapped within a 0.2 cM region flanked by markers *6A13699/6A13791/6A14397* and *6A14825* (Fig. 4c). These markers delimited a 4.3 Mb region (144.0–148.3 Mb) on the 6A pseudomolecule, resulting in an estimated 21.5 Mb/cM ratio of physical to genetic distance (Fig. 5a).

Twenty-nine high-confidence genes have been annotated in the 4.3 Mb candidate region in the IWGSC RefSeq v1.0 wheat genome (Supplementary Table S5) including several putative transcription factors (TFs) known to play important roles in plant development. These include *SQUAMOSA*

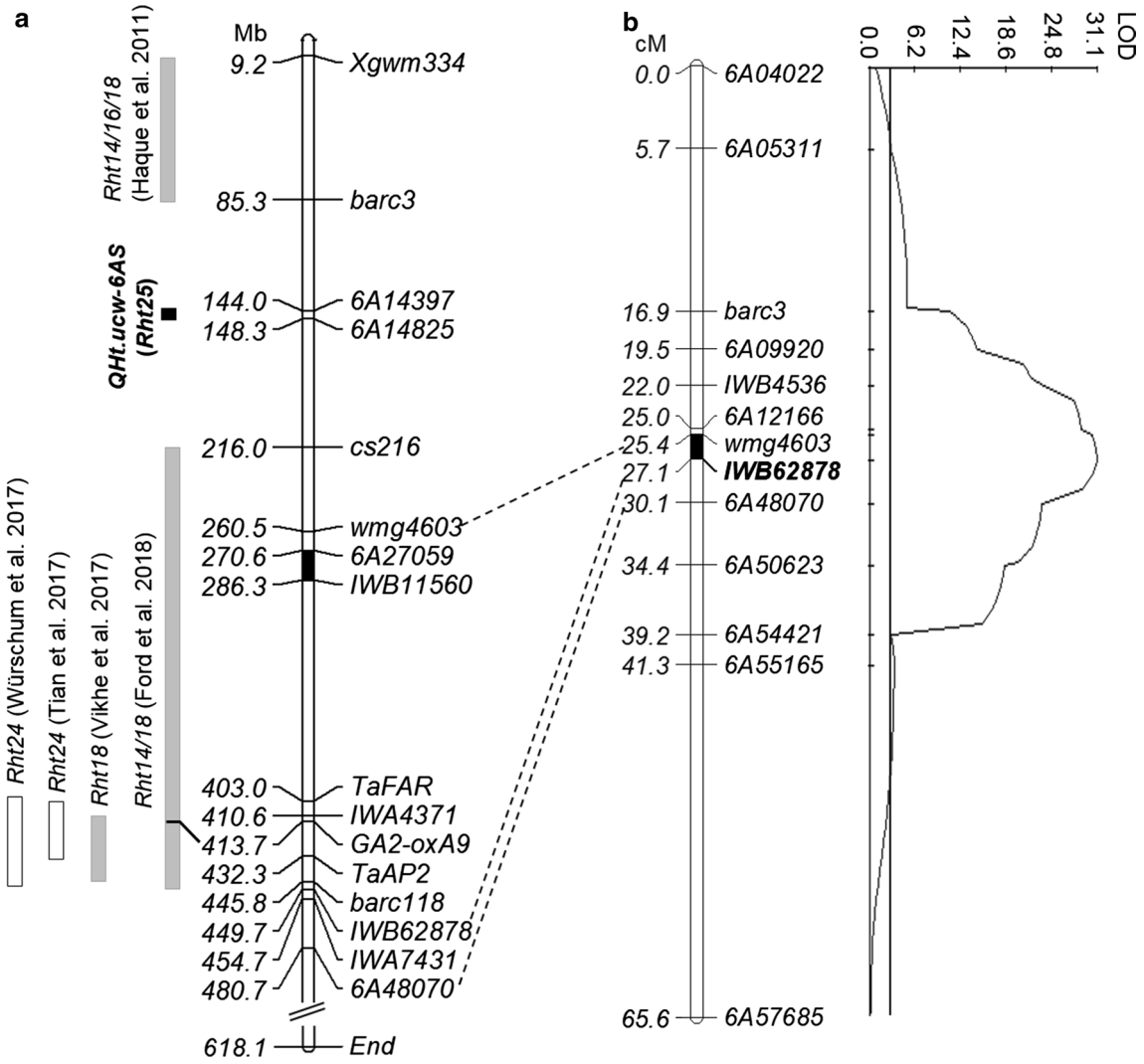

Fig. 5 Linkage map of the *Rht18* region and its comparison with other PH loci on chromosome 6A physical map. **a** Pseudomolecule 6A (IWGSC RefSeq v1.0) with the locations of *Rht18* (gray bars) and *Rht24* (white bars) determined from previous studies and *QHt.ucw-6AS* from this study (black bar). **b** *Rht18* linkage map from the Icaro/Langdon F_2 population. The peak marker (*IWB62878*) is highlighted in bold. A LOD plot is on the right side of the map, where the threshold (LOD = 3.0) line is indicated as a straight line. Centromere positions are shown as black boxes

promoter-binding-like TF TraesCS6A01G155300, MYB family TF TraesCS6A01G155400, ethylene-responsive TF TraesCS6A01G157300, and MADS-box TF TraesCS6A01G158100 (Chen et al. 2010; Dubos et al. 2010; Licausi et al. 2013; Pařenicová et al. 2003). We also found a PLATZ TF with unknown function (Nagano et al. 2001) and other genes that seem unlikely candidates for *QHt.ucw-6AS* (Supplementary Table S5).

The dominance effect of *QHt.ucw-6AS* on PH was evaluated using 1121 plants from 13 families segregating for this QTL (Supplementary Tables S2, S3, and S4; Supplementary Fig. S4a). The average PH difference between homozygous *QHt.ucw-6ASa* and *QHt.ucw-6ASb* plants was 11.9 cm, which was similar to the effect estimated from the original RIL population (9.1 cm). We calculated the degree of dominance for each of the 13 families segregating for PH as the difference between the average height of the heterozygotes and the midpoint value between the homozygotes, divided by the additive effect (Falconer 1960). The observed positive degree of dominance of 19.6 ± 7.7% indicates that the height-increasing allele *QHt.ucw-6ASa* is partially dominant (Supplementary Fig. S4a).

Rht18 mapping with the Icaro/Langdon F_2 population

We used 13 polymorphic markers on chromosome 6A to construct a genetic map for the F_2 population ($n = 120$)

derived from Icaro (*Rht18b*/*Rht-B1a*) crossed with Langdon (*Rht18a*/*Rht-B1a*), and conducted a QTL analysis for PH (Fig. 5b; Supplementary Fig. S4b). The peak of *Rht18* was mapped at *IWB62878* (27.1 cM), flanked by markers *wmg4603* (25.4 cM) and *6A48070* (30.1 cM). This region corresponded to a 220.2 Mb region (260.5–480.7 Mb) on pseudomolecule 6A (IWGSC RefSeq v1.0; Fig. 5a). The estimated ratio of physical to genetic distance for this region was 73.4 Mb/cM, reflecting its centromeric location and relatively low recombination frequency.

In the F_2 population, the average PH difference between homozygous *Rht18a* and *Rht18b* plants was 30.6 cm (Supplementary Fig. S4c). The degree of dominance for *Rht18* was estimated to be – 75.5%, indicating that the dwarfing allele *Rht18b* is partially dominant.

Discussion

QHt.ucw-6AS (*Rht25*) and other *Rht* genes on chromosome 6A

The Wheat Catalogue of Gene Symbols (McIntosh et al. 2017) list four named *Rht* genes on chromosome 6A. *Rht14*, *Rht16*, and *Rht18* are mutagenesis-induced alleles in durum wheat cultivars. *Rht14* in 'Castelporziano' (PI 347731) was induced by thermal neutron mutagenesis of 'Capelli', *Rht16* in 'Edmore M1' (PI 499362) was induced by methylnitrourea mutagenesis of 'Edmore', and *Rht18* in 'Icaro' (PI 503555) was induced by fast neutron mutagenesis of 'Anhinga' (McIntosh et al. 2017). *Rht24* is a natural allele that is present in many Chinese hexaploid wheat cultivars (McIntosh et al. 2017; Tian et al. 2017; Würschum et al. 2017).

The dwarfing alleles of these four named *Rht* genes on chromosome 6A are GA-responsive (McIntosh et al. 2017), a characteristic also demonstrated in this study for *QHt.ucw-6AS*. Ford et al. (2018) have recently shown that the PH reduction by *Rht18* was associated with increased expression of the gene encoding GA 2-oxidaseA9 (*GA2ox-A9*), and that mutations in the coding region of this gene eliminated the semi-dwarf phenotype of the *Rht18* mutants. The upregulation of *GA2ox-A9* increases the synthesis of inactive forms of GA and reduces the amount of the active forms of GA. Interestingly, *GA2ox-A9* maps within the *Rht14/18* region (Fig. 5a), suggesting that it might be a candidate gene for *Rht18* (Ford et al. 2018).

The comparison of the chromosome location of *Rht14, Rht16*, *Rht18*, and *Rht24* with *QHt.ucw-6AS* is presented in Fig. 5a, where the published genetic markers were translated into 6A pseudomolecule coordinates (IWGSC RefSeq v1.0) to facilitate comparisons. The early maps from Haque et al. (2011) show some inconsistencies with the more recent maps. Haque et al. (2011) mapped *Rht14*, *Rht16*, and *Rht18* on chromosome arm 6AS distal to microsatellite marker *barc3* (Fig. 5a). However, these results should be considered with caution because the *Rht* genotypes were inferred from phenotypes of individual F_2 plants without progeny tests (94 F_2 plants per population), and the maps included a limited number of markers (9–10 SSR loci each). In spite of these limitations, the study of Haque et al. (2011) provided valuable information on the allelic relationships among these genes. In three independent allelism tests including 152–158 F_2 plants each, no tall plant was detected indicating that *Rht14, Rht16*, and *Rht18* are allelic.

In this study, we mapped *Rht18* 10.2 cM proximal to *barc3* (Fig. 5b), which agrees with the *Rht18* locations on chromosome arm 6AL reported by Ford et al. (2018) and Vikhe et al. (2017) (Fig. 5a). These last studies confirmed that *Rht14* and *Rht18* are allelic, as reported by Haque et al. (2011). The *Rht14/Rht18* candidate region on chromosome arm 6AL (Ford et al. 2018; Vikhe et al. 2017) overlaps with the *Rht24* candidate region identified in two independent studies, one using a RIL population (Tian et al. 2017) and the other one using a world-wide association mapping panel (Würschum et al. 2017). More precise mapping studies (or the cloning of the genes) will be required to test if *Rht14*, *Rht16*, *Rht18*, and *Rht24* represent the alleles of a same gene or are tightly linked genes (Fig. 5a). Since *QHt.ucw-6AS* was mapped in a region of the short arm that does not overlap with any of the previously named *Rht* genes on chromosome 6A (Fig. 5a), we concluded that *QHt.ucw-6AS* is a different gene.

QHt.ucw-6AS also differs in other characteristics from the previously named *Rht* genes. In this study, we showed that *QHt.ucw-6AS* was semi-dominant for the height-increasing allele ($19.6 \pm 7.7\%$; Supplementary Fig. S4a), whereas *Rht14, Rht16,* and *Rht18* were reported to be semi-dominant for the dwarfing alleles (Haque et al. 2011; Konzak 1988). We confirmed here that *Rht18* is semi-dominant for the dwarfing allele (degree of dominance = – 75.5%, Supplementary Fig. S4c). In addition, *QHt.ucw-6AS* has a significant pleiotropic effect on coleoptile length, which was not observed for *Rht18* (Vikhe et al. 2017). Different pleiotropic effects on GW were also observed for *QHt.ucw-6AS* (Table 2) and *Rht24* (Tian et al. 2017).

Taken together, these observations indicate that *QHt.ucw-6AS* is different from the *Rht* genes previously mapped on chromosome 6A and was officially assigned the *Rht25* name.

Rht25 and other PH QTLs on chromosome 6A

Four QTL for PH have been mapped on the proximal region of chromosome 6A: *QHt.ipk-6A* (*cdo270-fba234*) (Börner et al. 2002), *QHt.fcu-6AS* (*barc23–fcp201*) (Liu et al. 2005), *QHt*-6A (peak marker *NW3106*) (Spielmeyer et al. 2007),

and *QHt-6A_1* (*wmc182–psp3029*) (Griffiths et al. 2012). These QTL encompass large chromosome regions, and it was not possible to find genomic coordinates for most of the reported flanking markers. Therefore, we were not able to compare the map location of these QTL with the locations of *Rht25* and the other named *Rht* genes on chromosome 6A (Fig. 5a).

Similar to the pleiotropic effects of *Rht25* observed in the present study, the height-increasing allele of *QHt.ipk-6A* (Börner et al. 2002) was associated with longer spike length, and the height-increasing allele of *QHt-6A* (Spielmeyer et al. 2007) was associated with longer coleoptile length. By contrast, *QHt_6A_1* (Griffiths et al. 2012) exhibited a pleiotropic effect on heading date in opposite direction to the one observed here for *Rht25* (i.e., the dwarfing allele was associated with later heading for *QTL-height_6A_1*, in contrast to earlier heading for *Rht25*). These results suggest that *QHt.ipk-6A* (Börner et al. 2002) and *QHt-6A* (Spielmeyer et al. 2007) are more likely to represent the same locus as *Rht25* than *QHt_6A_1* (Griffiths et al. 2012). The cloning of *Rht25* and its characterization in the parental lines used to map the four 6A QTL for PH, or more precise maps of these four QTL will be required to determine whether they represent the same or linked gene. We have initiated a detailed analysis of the 29 high-confidence genes present in the *Rht25* candidate gene region (Supplementary Table S5).

Rht25b as an alternative dwarfing source for fine-tuning PH

The majority of modern wheat cultivars carry either *Rht-B1b* or *Rht-D1b* as a major dwarfing source. The wide-spread utilization of these alleles is attributed to their beneficial effects on grain yield by improving harvest index and preventing lodging under optimal environments (Evans 1998; Guedira et al. 2010; Youssefian et al. 1992). However, height reduction by *Rht-B1b* or *Rht-D1b* can be detrimental under adverse conditions such as hot and/or dry climates or low-input production systems. In such cases, adverse effects on yield can occur due to decreased biomass and incomplete grain filling (Kertesz et al. 1991; Laperche et al. 2008; Richards 1992; Worland 1986). Producers in such sub-optimal environments may benefit from using alternative dwarfing alleles with a milder height-reducing effect and greater biomass production. The dwarfing effect of *Rht25b* (− 11.6%) is approximately half that of *Rht-B1b* (− 20.4%) and *Rht-D1b* (− 24.9%; Table 2), suggesting that it may serve as a useful alternative dwarfing source in sub-optimal environments.

Dwarfing alleles conferring effects of different magnitudes can be useful for fine-tuning PH to an optimum level in diverse genetic backgrounds and environments. For example, *Rht-B1b* or *Rht-D1b* can be combined with *Rht25b* to obtain a shorter stature, or with *Rht25a* to develop 'tall dwarf' cultivars which carry one of the major dwarfing alleles (*Rht-B1b* or *Rht-D1b*) for lodging tolerance along with height-increasing alleles of other PH loci for increased biomass (Law et al. 1978). However, the effect of PH genes is dependent on the environment and, therefore, long-term studies of the different allelic combinations of *Rht-B1*, *Rht-D1*, and *Rht25* will be required to determine the optimal combinations for each environment.

The close proximity (~ 10 cM) of *Rht25* and *Rht14/18* opens the possibility of stacking the dwarfing alleles of these two genes to take advantage of their combined effect. If this effect is favorable in a particular environment, the two genes can be transferred as a single block using markers flanking both genes. The precise mapping of *Rht25* in this study (0.2 cM) and of *Rht18* in recent studies (Ford et al. 2018; Vikhe et al. 2017) will facilitate the identification of recombination events combining dwarfing alleles of both genes. We have already initiated crosses to achieve this objective.

Rht25 (alone or in combination with *Rht18*) can be further combined with other minor *Rht* genes. Previous reports showed that *Rht8b* also confers a relatively mild dwarfing effect similar to *Rht25b*, reducing PH by ~ 7% (Rebetzke et al. 2012, Lanning et al. 2012). Because *Rht8* reduces PH without affecting coleoptile length, it provides an alternative dwarfing source that enhances early seedling vigor and improves crop establishment under deep sowing conditions (Rebetzke and Richards 2000, Rebetzke et al. 2007). Since the negative effect on coleoptile length of *Rht25b* (− 4.5%) is milder than that of *Rht-B1b* (− 17.1%; Fig. 3a), the combination of *Rht25* and *Rht8* may still provide some benefits for deeper planting. As different GA-sensitive PH genes and QTL are being identified and mapped, a more detailed characterization of their epistatic interactions in different genetic backgrounds and environments will be required to inform their deployment in wheat breeding programs.

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Author contribution statement LSV and IH developed mapping populations and conducted initial QTL mapping experiments. EJS and FG conducted field experiments and QTL analyses. YM, JA, and NO conducted high-resolution mapping experiments and GA sensitivity essays. YM and LSV wrote the first manuscript. SP and MH contributed to data analyses and manuscript revision. JD initiated and coordinated the project, contributed to data analyses, provided extensive revision and wrote the final manuscript. All authors reviewed the manuscript and provided suggestions.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval This study does not include human or animal subjects.

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