Theor Appl Genet (2015) 128:865–874
DOI 10.1007/s00122-015-2476-2

ORIGINAL PAPER

Genetic control of plant height in European winter wheat cultivars

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Received: 3 November 2014 / Accepted: 4 February 2015 / Published online: 17 February 2015

Abstract

Key message **Plant height variation in European winter wheat cultivars is mainly controlled by the *Rht-D1* and *Rht-B1* semi-dwarfing genes, but also by other medium- or small-effect QTL and potentially epistatic QTL enabling fine adjustments of plant height.**

Abstract Plant height is an important goal in wheat (*Triticum aestivum* L.) breeding as it affects crop performance and thus yield and quality. The aim of this study was to investigate the genetic control of plant height in European winter wheat cultivars. To this end, a panel of 410 winter wheat varieties from across Europe was evaluated for plant height in multi-location field trials and genotyped for the candidate loci *Rht-B1*, *Rht-D1*, *Rht8*, *Ppd-B1* copy number variation and *Ppd-D1* as well as by a genotyping-by-sequencing approach yielding 23,371 markers with known map position. We found that *Rht-D1* and *Rht-B1* had the largest effects on plant height in this cultivar collection explaining 40.9 and 15.5 % of the genotypic variance, respectively, while *Ppd-D1* and *Rht8* accounted for 3.0 and 2.0 % of the variance, respectively. A genome-wide scan for marker–trait associations yielded two additional medium-effect QTL located on chromosomes 6A and 5B explaining 11.0 and 5.7 % of the genotypic variance after the effects of the candidate loci were accounted for. In addition, we identified several small-effect QTL as well as epistatic QTL contributing to the genetic architecture of plant height. Taken together, our results show that the two *Rht-1* semi-dwarfing genes are the major sources of variation in European winter wheat cultivars and that other small- or medium-effect QTL and potentially epistatic QTL enable fine adjustments in plant height.

Communicated by I. Mackay.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2476-2) contains supplementary material, which is available to authorized users.

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Introduction

Plant height is an important trait in wheat (*Triticum aestivum* L.) breeding as it is a key parameter affecting lodging and thus grain yield and grain quality. Consequently, the reduction of plant height has been an important breeding goal in the last decades (Griffiths et al. 2012). A reduction of crop height can be associated with reduced grain yield (Law et al. 1978) and the aim in breeding programs is therefore the identification of variants that reduce height without adversely affecting yield. Thus, understanding the genetic control of plant height is key to a knowledge-based improvement of this trait.

The identification of major dwarfing or semi-dwarfing genes was an important step and nowadays enables plant breeders to modify plant height in wheat. The introduction of these so-called *Reduced height* (*Rht*) genes into wheat was also a major component of the 'green revolution' (Hedden 2003). Height-reducing alleles of *Rht-B1* and *Rht-D1* are the major sources of semi-dwarfism in current wheat breeding material and actually increase grain yield in most environments (Flintham et al. 1997). The *Rht-1* homoeoloci, which are located on group 4 chromosomes, encode DELLA proteins that integrate hormonal (gibberellin) and environmental signals to act on plant growth (Archard et al. 2006; Wilhelm et al. 2013a). For *Rht-B1*, two novel

alleles were recently identified that contain insertions of 160 or 197 bp upstream of the coding region and which also reduced plant height (Wilhelm et al. 2013b). Two additional *Rht* loci, *Rht7* and *Rht8*, were identified on chromosomes 2A and 2D, respectively, and may thus represent a homeologous series (Börner et al. 1996; Korzun et al. 1998). In addition, the photoperiod-insensitive alleles of the major photoperiod regulator *Ppd-1*, located on group 2 chromosomes, have also been reported to exert pleiotropic effects on plant height in wheat (Börner et al. 1993).

In addition to these major *Rht* genes, quantitative trait locus (QTL) mapping studies have identified a number of QTL for plant height in wheat (e.g., Cadalen et al. 1998; Kato et al. 1999). Griffiths et al. (2012) have recently performed a meta-QTL study based on four winter wheat doubled haploid populations which identified 16 meta-QTL. In addition, association mapping approaches have recently been adopted for QTL detection in plants (Würschum et al. 2012) and have also been applied in wheat (e.g., Rousset et al. 2011; Reif et al. 2011a, b; Le Couviour et al. 2011; Le Gouis et al. 2012; Kollers et al. 2013). In contrast to classical linkage mapping which is based on biparental families, association mapping is based on a panel of lines with different degrees of relatedness.

Despite the existing studies on plant height in wheat, the effects of key candidate loci and additional loci required to fine-tune plant height in European winter wheat remain less clear. The aim of this study was, therefore, to investigate the genetic architecture underlying variation of crop height in European winter wheat cultivars. To this end, a panel of 410 varieties was evaluated in multi-location field trials and genotyped at candidate loci and by a genotyping-by-sequencing approach. In particular, our objectives were to (i) assess the effect of the candidate genes *Rht-B1*, *Rht-D1*, *Rht8* as well as *Ppd-B1* copy number variation and *Ppd-D1* on plant height under field conditions, (ii) perform a genome-wide association study to identify additional loci affecting plant height in this cultivar collection, (iii) evaluate the contribution of epistasis and (iv) investigate the frequency of the candidate genes and identified major QTL dependent on the country of origin of the varieties.

Materials and methods

Plant materials, field experiments and meteorological data

A total of 410 soft winter wheat (*Triticum aestivum* L.) lines were used for this study as described by Langer et al. (2014). In brief, genotypes were European cultivars mainly originating from Austria, Czech Republic, Denmark, Eastern Europe, France, Germany, Poland, Russia, Turkey and the UK. Experiments were conducted in observation plots of two rows and 1.25 m length at three locations in 2012 in partially replicated designs with a replication rate of 1.27 per location (Williams et al. 2011). Locations were Hohenheim (HOH, 48°42′50″N, 9°12′58″E, 400 m above sea level (asl), growing season mean temperature 9.6 °C and mean precipitation 790 mm, soil type silty loam), Ihinger Hof (IHO, 48°44′50″N, 8°55′18″E, 493 m asl, growing season mean temperature 8.7 °C and mean precipitation 923 mm, soil type silty clay) and Oberer Lindenhof (OLI, 48°28′26″N, 9°18′12″E, 700 m asl, growing season mean temperature 7.4 °C and mean precipitation 1,115 mm, soil type silty loam). Plant height was recorded at full maturity and measured in centimeters from the ground to the tip of the spikes, excluding awns.

Molecular data analysis and candidate genes

All lines were genotyped by genotyping-by-sequencing (GBS) at Diversity Arrays Technology (Yarralumla, Australia) using the Wheat GBS 1.0 assay. The markers were subjected to the following quality checks before further analyses: markers with more than 25 % missing values and those with a minor allele frequency smaller than 0.05 were removed resulting in a total of 23,371 markers for which a map position was available.

For the candidate gene approach, all lines were genotyped for alleles of *Rht-B1*, *Rht-D1*, *Rht8* and *Ppd-B1* copy number variation and *Ppd-D1* (Liu et al. 2012; Kamran et al. 2014). Protocols for reduced height alleles causing semi-dwarfism (*Rht-B1b*, *Rht-D1b*) and tall alleles (*Rht-B1a*, *Rht-D1a*) were reported by Ellis et al. (2002) and by Wilhelm et al. (2013a) for *Rht-B1a* insertions (*Rht-B1a-0*, no insertion; *Rht-B1a-160*, 160 bp insertion; *Rht-B1a-197*, 197 bp insertion). The plants were genotyped with the microsatellite marker WMS 261 whose 192 bp allele can in some germplasm be diagnostic for the *Rht8* dwarfing allele (Korzun et al. 1998; Ellis et al. 2007). Copy numbers for *Ppd-B1* (*Ppd-B1a*, *Ppd-B1b*, *Ppd-B1c*, *Ppd-B1d* and *Ppd-B1e* indicating 3, 1, 4, 2 and 0 copies, respectively) were detected following the protocol described by Díaz et al. (2012) using, however, [6FAM-BHQ1] and [CY5-BHQ3] labeled probes for *Ppd-B1* and *TaCO2*, respectively, and the Roche LightCycler® 480 System in combination with the Roche LightCycler® 480 Probes Master mastermix. The photoperiod-insensitive allele *Ppd-D1a* (candidate causal deletion of 2,089 bp upstream coding region, 'Ciano67' type) and photoperiod-sensitive *Ppd-D1b* were detected following the method described by Beales et al. (2007). The allele status of the varieties for these loci and the phenotypic values are provided as Supplementary file 1.

Phenotypic data analysis

The phenotypic data were analyzed based on the following statistical model: $y_{ijko} = \mu + g_i + l_j + gl_{ij} + r_{jk} + b_{jko} + e_{ijko}$,

where y_{ijko} was the phenotypic observation of the ith wheat line at the jth location in the oth incomplete block of the kth replication, μ was an intercept term, g_i the genetic effect of the ith genotype, l_j the effect of the jth location, gl_{ij} the genotype-by-location interaction, r_{jk} the effect of the kth replication at the jth location, b_{jko} the effect of the oth incomplete block of the kth replication at the jth location and e_{ijko} the residual. Error variances were assumed to be heterogeneous among locations. Variance components were determined by the restricted maximum likelihood (REML) method assuming a random model and model comparison with likelihood ratio tests were used to test the significance of the variance component estimates. For subsequent analyses, best linear unbiased estimates (BLUEs) were estimated across locations for all genotypes. Heritability (h^2) was estimated following the approach suggested by Piepho and Möhring (2007). All statistical analyses were performed using ASReml 3.0 (Gilmour et al. 2009).

Association mapping

For association mapping, an additive genetic model was chosen and mapping was done with a mixed model incorporating a kinship matrix as described previously (Würschum and Kraft 2014a; Langer et al. 2014) and controlling for multiple testing, with a false discovery rate (FDR) of 0.20 (Benjamini and Hochberg 1995). In addition, we employed a modified multi-locus model for detection of main-effect QTL that has recently been shown to be competitive with the commonly used kinship matrix model and may perform better for traits with a genetic architecture including some major-effect QTL (Würschum and Kraft 2014b). The modification was the inclusion of a random genotypic effect (without kinship matrix) in the model, while cofactor selection was done as described. The two-dimensional epistasis scan was done based on 2,594 equally spaced markers including the detected main-effect QTL, by extending the mixed model to include marker–marker interactions including the respective main effects. For the significance level for the epistatic QTL we used a Bonferroni-corrected α level of 0.01. The circular plots illustrating the epistatic interactions were created with Circos (Krzywinski et al. 2009).

The total proportion of genotypic variance (p_G) explained by the detected QTL was calculated by fitting the segregating candidate genes and all QTL simultaneously in a linear model to obtain R^2_{adj}. The ratio $p_G = R^2_{adj}/h^2$, where h^2 refers to the heritability of the trait, yielded the proportion of genotypic variance (Utz et al. 2000). The p_G values of individual QTL were accordingly derived from the sums of squares of the QTL (SS_{QTL}) in this linear model. For the fivefold cross-validation the mapping population was divided into an estimation set comprising 80 % of the lines and a test set with the remaining 20 % of the lines (Würschum and Kraft 2014a). The effects of the candidate genes and the QTL detected in the full data set were estimated in the estimation set and used to predict the genotypic values of the lines in the test set. The proportion of genotypic variance explained by these loci in the independent test set was calculated from the R^2_{adj} between the observed and the predicted values of the lines in the test set, divided by the heritability of the trait. The presented result is the mean value from 10,000 cross-validation runs.

Results

The 410 winter wheat genotypes were evaluated in partially replicated designs at three locations. We observed significant ($P < 0.01$) genotypic and genotype-by-location interaction variances (Table 1) and obtained a high heritability of 0.85. The adjusted entry means for plant height ranged from 57.9 to 121.6 cm with a mean of 76.1 cm. The histogram illustrates that most genotypes have a plant height around the mean with only few being taller than 100 cm (Fig. 1, Figure S1).

The candidate genes *Rht-B1*, *Rht-D1*, *Rht8* and *Ppd-B1* copy number variation (CNV) and *Ppd-D1* were all polymorphic in the studied panel of wheat lines allowing to evaluate the effects of these genes on plant height in European winter wheat germplasm. The strongest effect on plant height was observed for *Rht-D1* (Fig. 1) as this gene explained the highest proportion of genotypic variance with 40.9 % and the allele substitution effect of the semi-dwarfing *Rht-D1b* allele was −5.7 cm (Table 2, Table S1). The second largest proportion of genotypic variance was attributable to *Rht-B1* with 15.5 %. The combination of both semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b*, in double dwarf lines resulted in a further reduction of plant

Table 1 Summary statistics for plant height (cm)

Parameter	Across locations	Location HOH	Location IHO	Location OLI
Min	57.9	61.5	53.3	53.2
Mean	76.1	80.0	73.8	74.8
Max	121.6	126.8	113.8	118.1
σ^2_G	117.6**	133.6**	113.1**	148.5**
$\sigma^2_{G\times L}$	4.0**			
σ^2_L	11.1**			
σ^2_e	22.6	12.9	14.8	25.2
h^2/R	0.85	0.84	0.83	0.80

Genotypic variance ($\sigma_G{}^2$), genotype-by-location interaction variance ($\sigma^2_{G\times L}$), location variance ($\sigma_L{}^2$), error variance ($\sigma_e{}^2$) and heritability (h^2) or repeatability per location (R)

** Significant at the 0.01 probability level

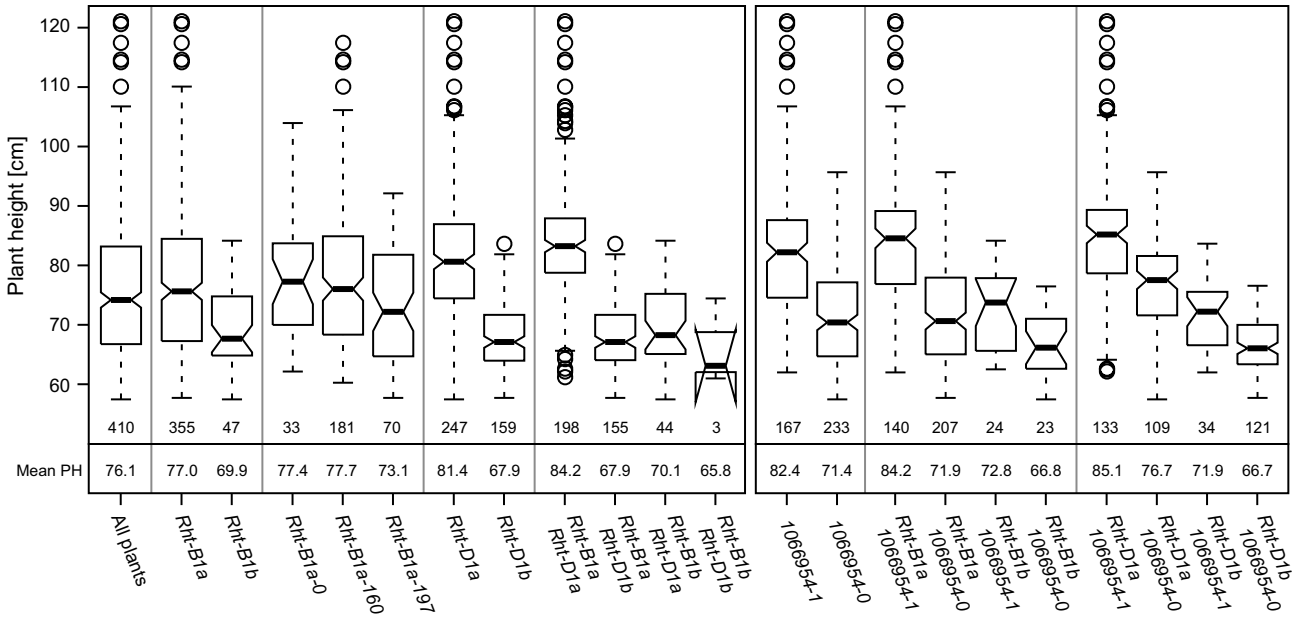

Fig. 1 *Box plots* showing plant height for genotypes carrying different alleles of the two *Rht-1* candidate genes or the detected QTL (QTL-6A, CloneID 1066954) as well as combinations thereof. The numbers underneath the *box plots* indicate the number of genotypes in each group. The *box plots* show the median as *thick line* and the 1 and 3 quartile as *box*, and the lowest and highest value still within the 1.5 interquartile ranges as whiskers; non-overlapping notches indicate statistically significant differences between the medians. Mean PH is the mean plant height in each group; *Rht-B1* and *Rht-D1 a* (tall allele), *b* (semi-dwarfism)

height compared to plants only homozygous for one of the two alleles. Consequently, the double dwarfs were the shortest genotypes in our panel with a mean plant height of 65.8 cm. Of the two additional *Rht-B1* alleles containing insertions of 160 or 197 bp upstream of the coding region, the *Rht-B1a-160* allele was present in 189 of the accessions and the *Rht-B1a-197* allele in 70 of the accessions. With few exceptions, these two *Rht-B1* insertions occurred in accessions carrying the *Rht-B1a* allele and their effects were therefore estimated relative to *Rht-B1a-0*, i.e., the *Rht-B1a* allele without additional insertion. *Rht-B1a-160* had no effect, while *Rht-B1a-197* had a small effect and explained 2.6 % of the genotypic variance within *Rht-B1a* plants.

The 192 bp allele at the microsatellite marker WMS 261 has initially been thought to be diagnostic for the dwarfing gene *Rht8* (Korzun et al. 1998), but Ellis et al. (2007) showed this association to be strongly dependent on the germplasm. In our panel of wheat cultivars, this allele had only a small effect on plant height and explained only 2.0 % of the genotypic variance (Figure S2). The major photoperiod locus in wheat, *Ppd-D1*, also affected plant height (Figure S3). The photoperiod-insensitive *Ppd-D1a* allele resulted in reduced height, even in the background of the semi-dwarfing *Rht-B1b* or *Rht-D1b* alleles. *Ppd-D1* explained 3.0 % of the genotypic variance and the allele substitution effect of the photoperiod-insensitive allele was −1.5 cm (Table 2). In addition, we evaluated the effect of copy number variation at the *Ppd-B1* locus and found this to have only a minor effect on plant height (Figure S4).

The 410 winter wheat varieties were genotyped by a genotyping-by-sequencing approach. After quality checks 23,371 polymorphic markers with known map position remained which were used for association mapping. The genome-wide scan for marker–trait associations identified 21 markers that were significantly associated with plant height after correcting for multiple testing (Fig. 2; Table 2, Table S1). Two markers were identified on chromosome 4D which were likely detected as they are in linkage disequilibrium with *Rht-D1*. This collinearity with *Rht-D1* was substantiated, as in a linear model with *Rht-D1* they did not explain any genotypic variance. Of the remaining seven QTL only three explained more than 1 % of the genotypic variance: the QTL on chromosome 6A with 11.0 %, on chromosome 5B with 5.7 % and on chromosome 7D explaining 2.2 %. The strongest of these three QTL, located on chromosome 6A, was identified by 13 collinear markers (Fig. 2). This QTL (CloneID 1066954) also resulted in a substantial height reduction and even reduced plant height in plants already homozygous for the semi-dwarfing *Rht-B1b* or *Rht-D1b* alleles (Fig. 1). A similar picture was observed for the second largest of the detected QTL (CloneID 1250057), except that this QTL only reduced plant height in the background of the

Table 2 Candidate genes and QTL detected for plant height

Marker/CloneID	Chr.	Pos.	P_G	α effect	$R_{Rht\text{-}D1}$
Candidate genes					
Rht-B1	4B		15.5	−5.0	
Rht-B1a 160 bp ins.	4B		0.0[a]		
Rht-B1a 197 bp ins.	4B		2.6[a]	−2.0[a]	
Rht-D1	4D		40.9	−5.7	
Rht8 (192 bp allele)	2D		2.0	0.1	
Ppd-B1 CNV	2B		1.1	−3.6	
Ppd-D1	2D		3.0	−1.5	
Genome-wide scan					
3022436	2A	17.5	0.3	0.8	
1038091	2A	55.6	0.9	1.0	
1077814	5A	132.3	0.6	1.1	
1066954	6A	94.8	11.0	3.4	
1250057	5B	101.9	5.7	5.5	
1185372	1D	169.1	0.5	0.7	
3027223	4D	32.8	0.6	−0.1	0.74
1201923	4D	41.5	0.0		0.68
1217443	7D	56.1	2.2	−1.9	

Chromosome, position (cM), proportion of genotypic variance explained by the QTL (p_G in %), allele substitution (α) effect (in cm) and the correlation with *Rht-D1* ($r_{Rht\text{-}D1}$)

[a] Within plants homozygous for *Rht-B1a*

semi-dwarfing *Rht-D1b* allele, but not in *Rht-B1b* plants (Figure S5). Together, the candidate loci and the identified QTL explained 82.7 % of the genotypic variance. We employed a fivefold cross-validation approach to obtain an asymptotically unbiased estimate of the proportion of genotypic variance explained by the candidate loci and the detected QTL. This revealed only a small relative bias of approximately 5 % in the estimation of this important parameter and cross-validated these loci still explained 78.6 % of the genotypic variance.

In addition to the commonly used kinship matrix approach, we also employed a multi-locus model to detect markers significantly associated with plant height in this panel of cultivars. This approach identified *Rht-D1*, *Rht-B1* and *Ppd-D1* as well as nine QTL on different chromosomes, including the major QTL on chromosome 6A (Table S2; Figure S6). Jointly, the identified markers explained 91.0 % of the genotypic variance. The genome-wide epistasis scan identified 21 epistatic QTL explaining between 0.0 and 3.0 % of the genotypic variance (Fig. 3; Table S3). The strongest interaction was found between two loci on chromosomes 1A and 2A.

For 379 of the winter wheat varieties, the country where they were registered was known and we used this information to assess the frequency of alleles at the two *Rht-1*

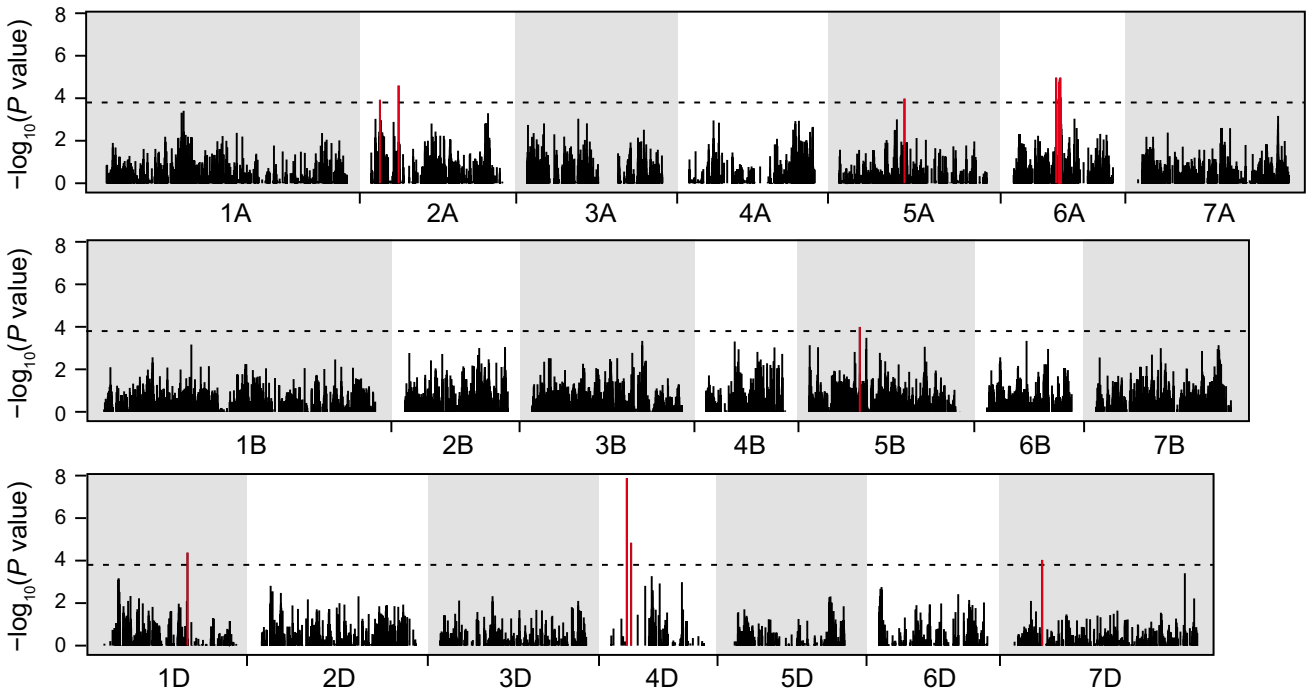

Fig. 2 Genome-wide scan for markers associated with plant height. The *dashed horizontal lines* indicate the significance threshold

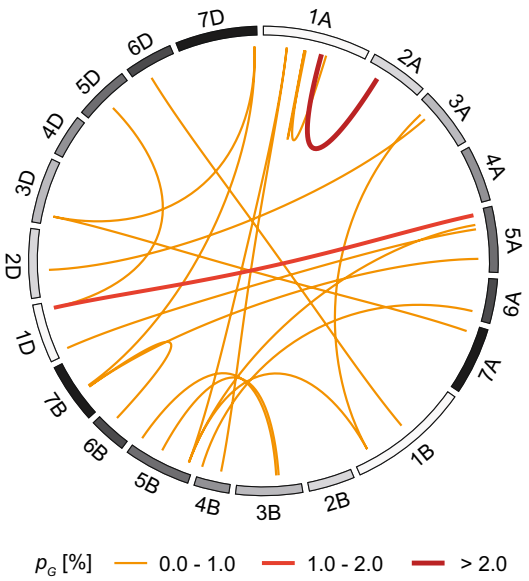

Fig. 3 Epistatic QTL for plant height

loci and of the two detected major QTL in different geographic regions (Fig. 4). This revealed that for *Rht-B1*, the height-reducing allele (*Rht-B1b*) was present at low frequency in Central European varieties and was slightly more frequent in varieties from Denmark, the UK and France. By contrast, more than half of the Eastern European varieties and all Turkish varieties carry this allele. For *Rht-D1*, the picture was reversed. About a quarter of the varieties from Central Europe carry the semi-dwarfing allele, around three-quarters of the lines from Denmark, the UK and France, but less than a quarter of the Eastern European and none of the Turkish lines. The geographic distribution of the height-reducing allele at the major QTL on chromosome 6A (1066954) somewhat mirrored the distribution of the *Rht-D1b* allele. About half of the lines from Central Europe carried this allele, more than three-quarters of the lines from Denmark, the UK and France, while in Eastern Europe where *Rht-D1b* was less frequent, this QTL allele was also less frequent or even absent as in Turkish lines. Of the 159 plants homozygous for *Rht-D1b*, 122 were also

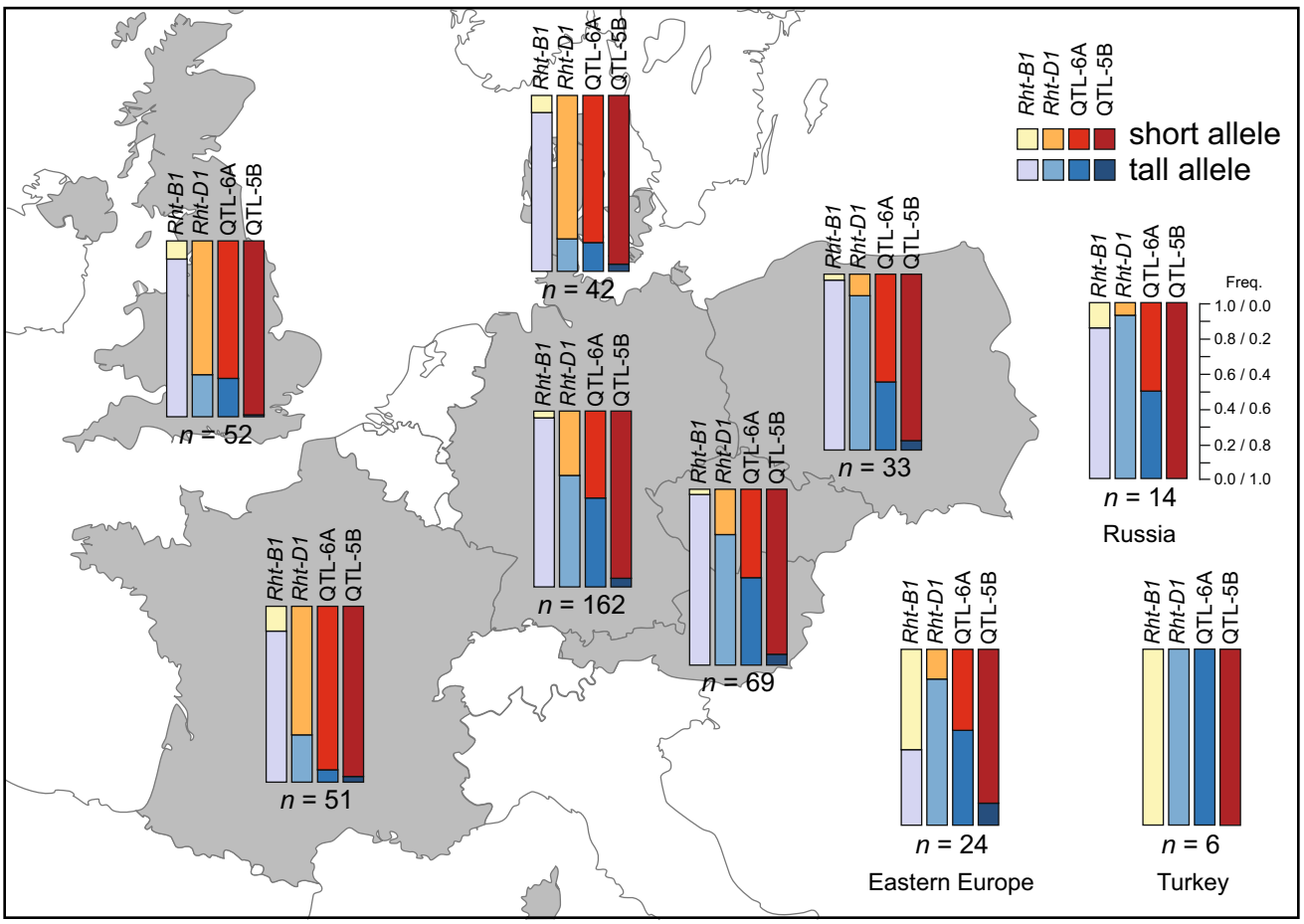

Fig. 4 Distribution of alleles of *Rht-B1*, *Rht-D1* and of the detected major QTL (QTL-6A: CloneID 1066954 and QTL-5B: 1250057) in European winter wheat dependent on the country in which the varieties are registered

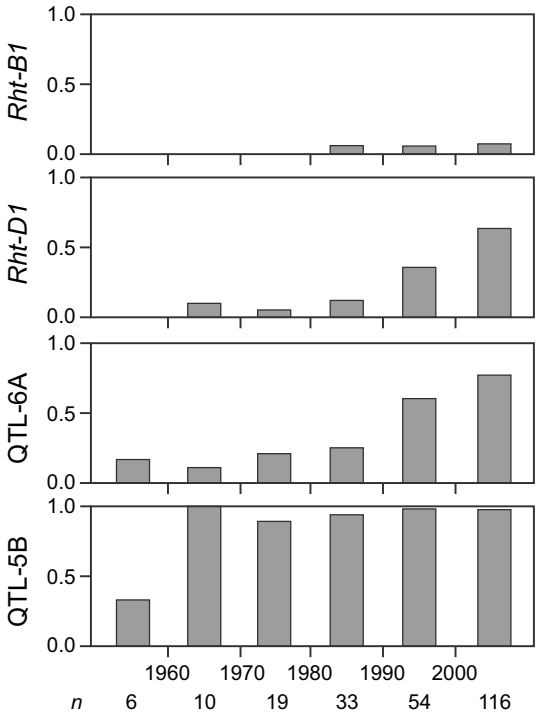

Fig. 5 Relative proportion of the height-reducing allele of *Rht-B1*, *Rht-D1* and of the detected major QTL (QTL-6A: CloneID 1066954 and QTL-5B: 1250057) dependent on the year of release of the varieties. The numbers underneath (*n*) indicate the number of plants for each time interval

homozygous for the height-reducing allele at this QTL on chromosome 6A (1066954). For the second detected major QTL (1250057), the height-reducing allele was the predominant allele in all geographic regions.

In addition to the geographic distribution of the alleles at these four loci, we also investigated the relative proportion of the height-reducing allele dependent on the year of release of the varieties (Fig. 5). This showed that the semi-dwarfing *Rht-D1b* allele is increasingly used since the 1970s and that the height-reducing allele at the major QTL on chromosome 6A (1066954) followed this development. The semi-dwarfing *Rht-B1b* allele is only rarely used in the varieties studied here, while the relative proportion of the height-reducing allele at the second major QTL on chromosome 5B (1250057) has remained constantly high in the last decades.

Discussion

Phenotypic evaluation of plant height in European winter wheat cultivars

The genotypic variance and the genotype-by-location interaction variance were both significant, but the genotypic variance was by far the larger of the two, which confirms previous findings (cf. Longin et al. 2013). This illustrates that this highly heritable trait is mainly controlled by the genotype which is promising for knowledge-based breeding. We observed a large range in genotypic values for plant height which is likely due to the broad geographic sampling of varieties from different regions within Europe, as well as from different registration periods. The median height was, however, only 74 cm illustrating that breeders have already strongly selected for reduced plant height. Different semi-dwarfing genes were used intensively since the green revolution making it worthwhile to investigate their effects and distribution in our collection of European cultivars as well as to study the genetic control of plant height in this panel.

Rht candidate genes in European winter wheat and their effects on plant height

The gibberellic acid (GA)-insensitive semi-dwarfing genes *Rht-B1b* and *Rht-D1b* are the best characterized crop height genes in wheat (Wilhelm et al. 2013a, b) and are estimated to be in approximately 90 % of the semi-dwarfing varieties worldwide (Worland et al. 1998). Wilhelm et al. (2013b) found the *Rht-B1b* and *Rht-D1b* alleles each to be present in 12.5 % of the accessions of their worldwide wheat panel. As pointed out by the authors, this relatively low proportion of *Rht-B1b* and *Rht-D1b* alleles was likely due to the inclusion of landraces and accessions pre-dating the incorporation of *Rht-1* semi-dwarfing alleles in breeding material which began in the 1960s. Le Couviour et al. (2011) observed different frequencies of the *Rht-B1b* and *Rht-D1b* alleles in lines from France, UK and Germany released in the last four decades, indicating a differential use of the semi-dwarfing alleles. Knopf et al. (2008) investigated their frequency in German winter wheat varieties and found *Rht-B1b* to be present in 6 % and *Rht-D1b* in 38 % of the varieties. This is in accordance with our results as we found *Rht-B1b* and *Rht-D1b* in 4 and 36 % of the German varieties, respectively. In contrast to Germany, both semi-dwarfing alleles were much more frequent in varieties from Denmark, the UK or from France. Particularly, *Rht-D1b* was present in more than 75 % of these varieties (Fig. 4) which was mirrored by their shorter average height (Figure S7). Interestingly, the varieties from Poland or the Czech Republic and Austria had a median height comparable to that of the German varieties (Figure S7) despite a lower frequency of the *Rht-1* semi-dwarfing alleles (Fig. 4). This indicates that additional loci affecting plant height are present in European cultivars and can be utilized in breeding programs. While reducing plant height through semi-dwarfing *Rht-1* alleles can increase the yield potential of varieties, this association may not hold true under Southern European environmental conditions due to a reduced

fertility of *Rht-B1b* or *Rht-D1b* carrying plants when exposed to higher temperatures prior to ear emergence (Worland and Snape 2001). Consequently, wheat breeding targeted to these regions may even have resulted in selection against the semi-dwarfing *Rht-1* alleles. Consistently, only few of the Eastern European and Turkish lines carried the strong *Rht-D1b* allele and height reduction under these conditions appears to be achieved through the weaker *Rht-B1b* allele or through other loci, for example the photoperiod-insensitive *Ppd-D1a* allele or *Rht8*.

In accordance with previous findings, the two major *Rht-1* semi-dwarfing alleles had the strongest effect on plant height explaining 40.9 and 15.5 % of the genotypic variance and each locus having an additive effect of approximately 5 cm (Table 2). It must be noted that the QTL effects estimated here are based on height measurements of plants not treated with plant growth regulators. Consequently, these QTL effects can be expected to be somewhat smaller when growth regulators are applied to the plants. Nevertheless, this illustrates that these two loci and the corresponding molecular markers are valuable tools for a knowledge-based breeding of plant height in European winter wheat breeding germplasm. However, despite their potential to increase yield, varieties carrying the semi-dwarfing *Rht-B1b* or *Rht-D1b* alleles have the disadvantage of a higher susceptibility to the destructive Fusarium head blight disease (Srinivasachary et al. 2008; Bürstmayr et al. 2012). This may be due to an indirect effect of plant height per se, but could also be caused by a pleiotropic effect of the *Rht* loci or by tight linkage to a susceptibility locus and warrants further research.

The two additional *Rht-B1* alleles containing insertions of 160 or 197 bp in the promoter region have been shown to reduce plant height by 18 and 12 %, respectively, in a worldwide collection of wheat varieties (Wilhelm et al. 2013b). While the *Rht-B1a-160* insertion had no effect on plant height in our panel of European winter wheat, *Rht-B1a-197* had a small effect within *Rht-B1a* plants, which substantiates the conclusion that these insertion alleles may enable fine adjustments in plant height (Wilhelm et al. 2013b).

Wilhelm et al. (2013b) also observed an effect of *Ppd-D1* on plant height with the photoperiod-insensitive *Ppd-D1a* allele reducing height. However, *Ppd-D1a* is in close linkage with the GA-sensitive semi-dwarf allele of *Rht8* (Worland et al. 1998; Gasperini et al. 2012). Most wheat varieties containing *Rht8* are therefore photoperiod insensitive and thus early flowering and are consequently prevalent in Eastern and Southern Europe (Worland et al. 1988). In a study based on recombinant inbred lines segregating at both loci, Gasperini et al. (2012) attributed a 13 % plant height reduction to the semi-dwarf *Rht8* allele and only a 3 % reduction to *Ppd-D1a*. By contrast, we observed that in our panel the effect of *Ppd-D1* was slightly larger than that of *Rht8*, which may be due to the composition of the panel or the test environments. It must be noted here, however, that the 192 bp allele at the microsatellite marker WMS 261, which has been taken as diagnostic for *Rht8* (Korzun et al. 1998), has recently been shown to not always be associated with this dwarfing gene (Ellis et al. 2007). Ellis et al. (2007) suggested that after the Green Revolution a second haplotype had been introduced in international germplasm with the result that in these lines the 192 bp allele is not indicative of *Rht8* anymore. Consequently, in the absence of a truly diagnostic marker for *Rht8*, the frequency and the effects of this locus in European winter wheat remain elusive.

We observed a strong increase of the semi-dwarfing *Rht-D1b* allele in European winter wheat since its introgression in the 1960s (Fig. 5). By contrast, *Rht-B1b* remained present at a low frequency since the 1980s. It must be noted, however, that this picture is somewhat biased as it is strongly dependent on the number of varieties from the different European regions with different preferences for the two *Rht-1* loci. Nevertheless, these findings are in accordance with Wilhelm et al. (2013b) who reported a significant effect of the registration period on plant height, as varieties registered after 1939 were substantially shorter than varieties registered earlier. This was true even when the effects of *Rht-B1*, *Rht-D1* and *Ppd-D1* were taken into account, suggesting that additional loci affect plant height in modern bread wheat relative to older germplasm.

Main and epistatic QTL for plant height

Despite the large proportion of genotypic variance explained by the candidate loci, there is still approximately half of the genotypic variance not accounted for by these major height regulators. We therefore performed a genome-wide association mapping to identify novel loci affecting plant height in European winter wheat cultivars. This identified two QTL on chromosomes 6A and 5B, explaining more than 5 % of the genotypic variance even when the effects of the *Rht-1* loci were simultaneously considered in the linear model (Table 2). This indicates that these loci act independently of the *Rht-1* loci and were not identified due to co-selection and consequent linkage disequilibrium between alleles at these loci and *Rht-1* alleles. While the QTL on chromosome 5B seems to be almost fixed in this cultivar collection, the QTL on chromosome 6A appears more interesting and was identified by two different association mapping approaches (Würschum and Kraft 2014b). Its frequency varies in different countries (Fig. 4) and approximately follows the usage of *Rht-D1b*. This suggests that this QTL is a major regulator for the fine-tuning of plant height in a *Rht-D1b* background. Griffiths et al.

(2012), performing QTL mapping in four doubled haploid families, also reported a QTL located in the centromeric region of chromosome 6A which was found in three of the families. In addition, a QTL on chromosome 5B was found in two of the families and a strong QTL on chromosome 2D which likely corresponded to *Rht8* (Griffiths et al. 2012). This QTL, however, only segregated in one of the four families suggesting that the QTL was fixed in the other families. We did not identify a QTL on chromosome 2D which is likely due to an insufficient marker density in this region as a previous scan for flowering time QTL failed to identify a QTL on this chromosome despite *Ppd-D1* being the major contributor to flowering time variation in this population (Langer et al. 2014). Similarly, no QTL was identified on chromosome 4B which harbors *Rht-B1*. We did not identify any other major- or medium-effect QTL, suggesting that they are either present but not detected due to an insufficient marker density or that the fine-tuning of plant height is achieved by many QTL with effects too small to be detected. It must be noted here that plant height is a dynamic trait that changes with time and multiple assessments of plant height in triticale have recently revealed the temporal dynamics of the genetic control underlying this trait (Würschum et al. 2014). Thus, QTL active at an earlier stage would also not be captured by this analysis.

Another source for this variation not accounted for by the main-effect QTL is epistasis. Epistasis refers to interactions between two or more loci in the genome (Carlborg and Haley 2004) and has recently been shown to contribute to the genetic architecture of complex traits in different crops including maize, wheat and rapeseed (Buckler et al. 2009; Reif et al. 2011b; Liu et al. 2012; Steinhoff et al. 2012; Würschum et al. 2013). We did identify epistatic QTL for plant height. While the effects of these epistatic interactions were small, their combined effects may contribute substantially to the genetic control of plant height. This illustrates that both main and epistatic effects contribute to the genetic architecture of plant height in European winter wheat cultivars.

Conclusions

In this study we used a panel of 410 lines to investigate the genetic control of plant height in European winter wheat cultivars. We show that the two semi-dwarfing loci *Rht-B1* and *Rht-D1* are the major sources of variation of plant height and present at different frequencies throughout Europe. A genome-wide association mapping scan identified few QTL with medium effects including a QTL on chromosome 6A that explains approximately 10 % of the genotypic variance and that is preferentially used in combination with *Rht-D1b*. Taken together, the two major *Rht-1* loci and a few other loci are promising sources for a knowledge-based fine-tuning of plant height in European winter wheat breeding programs either through marker-assisted selection or by genomic selection with weights for the important functional markers (Zhao et al. 2014).

Author contribution statement SML collected phenotypic data, TW performed the analyses, and TW and CFHL designed the study and wrote the paper.

Acknowledgments This research was funded by the Deutsche Forschungsgemeinschaft under grant number WU 658/1-1.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The authors declare that the experiments comply with the current laws of Germany.

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