Theor Appl Genet (2013) 126:2233–2243
DOI 10.1007/s00122-013-2130-9

ORIGINAL PAPER

Rht-1 and *Ppd-D1* associations with height, GA sensitivity, and days to heading in a worldwide bread wheat collection

Edward P. Wilhelm · Margaret I. Boulton · Nadia Al-Kaff · Francois Balfourier · Jacques Bordes · Andy J. Greenland · Wayne Powell · Ian J. Mackay

Received: 13 February 2013 / Accepted: 11 May 2013 / Published online: 31 May 2013

Abstract *Reduced height* (*Rht*)*-1* and *Photoperiod* (*Ppd*) have major effects on the adaptability of bread wheat (*Triticum aestivum*) to specific environments. *Ppd–D1a* is a photoperiod insensitive allele that reduces time to flowering. The gibberellin (GA) insensitive alleles *Rht–B1b* and *Rht–D1b* shorten plant stature and were important components of the 'green revolution'. Two additional *Rht–B1* alleles were recently identified that contain a 160 or 197 bp insertion upstream of the coding region and may affect plant height or GA sensitivity Wilhelm et al. (Theor Appl Gen doi:10.1007/s00122-013-2088-7, 2013b). We determined the frequency of the five alleles in a worldwide core collection of 372 wheat accessions (372CC) and estimated their effects on height, days to heading, and GA sensitivity when the collection was grown in pots outdoors or in the glasshouse. This revealed that each allele was widespread geographically with frequencies ranging from 0.12 to 0.25. *Ppd-D1a* was associated with significant ($p \leq 0.05$) reductions in days to heading and height relative to photoperiod sensitive *Ppd-D1b*. Relative to wild type, *Rht-B1b* and *Rht-D1b* each resulted in significant reductions in height (approximately 30 %) and GA sensitivity. The 160 and 197 bp alleles were associated with significant height reductions of 18 and 12 %, respectively, and with non-significant reductions in GA sensitivity relative to wild type. Two statistical methods were developed and used to estimate GA sensitivity of the 372CC accessions, but novel GA insensitive alleles were not identified. Further characterization of the *Rht-B1* insertion alleles is required, but our results suggest these may enable fine adjustments in plant height.

Abbreviations

3722CC	Worldwide core collection of 372 wheat accessions, which are held at INRA (French National Institute for Agricultural Research)
FM	Fitted mean
GA	Gibberellin
GAI	Gibberellin insensitive
GAS	Gibberellin sensitive

Communicated by M. E. Sorrells.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2130-9) contains supplementary material, which is available to authorized users.

E. P. Wilhelm (✉) · A. J. Greenland · W. Powell · I. J. Mackay
National Institute of Agricultural Botany, Huntingdon Rd., Cambridge CB3 0LE, UK
e-mail: edwilhelm@yahoo.com

Present Address:
W. Powell
Institute of Biological, Environmental, and Rural Sciences, Aberystwyth SY23 3DA, UK

E. P. Wilhelm · M. I. Boulton · N. Al-Kaff
John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

Present Address:
N. Al-Kaff
Taibah University, P.O. Box 344, The Road of Universities, Al-Madina Al-Munwarah, Kingdom of Saudi Arabia

F. Balfourier · J. Bordes
INRA, UMR 1095, Genetics, Diversity and Ecophysiology of Cereals, 63100 Clermont-Ferrand, France

F. Balfourier · J. Bordes
UBP, UMR 1095, Genetics, Diversity and Ecophysiology of Cereals, 63100 Clermont-Ferrand, France

GA trt diff	Gibberellin treatment difference (STFL length in the GA+ treatment minus STFL length in the GA− treatment)
Int	Intermediate gibberellin response
LSD	Least significant difference
NIL	Near isogenic line
PDM	Probability distribution mixture
Ppd	*Photoperiod*
r	Pearson's correlation coefficient
REMLMM	Restricted maximum likelihood mixed model
Rht	*Reduced height*
STFL	Seed to first ligule

Introduction

Alleles at the *Reduced height (Rht)-1* and *Photoperiod* (*Ppd*)-*D1* loci have a major influence on the adaptability of wheat to climate and modern agricultural practices. *Rht-B1* and *Rht-D1* are located on chromosomes 4B and 4D, respectively, in bread wheat (*Triticum aestivum*) and encode copies of the DELLA protein, a growth repressor that is normally degraded in the presence of gibberellin (GA). *Rht-B1b* and *Rht-D1b* each contain a single nucleotide polymorphism that introduces a premature stop codon (Peng et al. 1999). The resulting DELLA proteins have a reduced sensitivity to GA relative to DELLA proteins encoded by the wild type *Rht-B1a* and *Rht-D1a* alleles, thereby reducing height (Peng et al. 1999). Individually, *Rht-B1b* and *Rht-D1b* are associated with height reductions of approximately 14–17 % relative to the wild type (Flintham et al. 1997). The semi-dwarf varieties have decreased lodging and improved harvest index under intensive agriculture, and *Rht-B1b* and *Rht-D1b* were thereby important components of the 'green revolution' (Hedden 2003). *Ppd-D1* is located on chromosome 2D in bread wheat and is a member of the *pseudo-response regulator* (*PRR*) gene family (Beales et al. 2007). In *Arabidopsis thaliana,* the *PRR* gene family members are components of the circadian clock (Mizuno and Nakamichi 2005; Matsushika et al. 2007). The wheat *Ppd-D1a* allele contains a 2,089 bp deletion in the promoter region, which is associated with photoperiod insensitivity (Beales et al. 2007). Under field conditions in the United Kingdom, ear emergence of *Ppd-D1a* varieties was accelerated by 6–8 days when October-sown, relative to varieties containing the photoperiod sensitive allele *Ppd-D1b* (Worland et al. 1988). Early flowering associated with *Ppd-D1a* is beneficial in areas such as Southern Europe, allowing plants to mature prior to summer heat stress (Worland et al. 1998a).

The *Rht-1b* and *Ppd-D1a* alleles have a pronounced effect on wheat adaptation, but the ability to further modify plant height and flowering time should aid in adapting varieties to specific environments. Several *Ppd-D1* haplotypes, including *Ppd-D1a*, were recently assessed and the associated effects on flowering date and several agronomic traits suggest several haplotypes may be useful in fine-tuning wheat to specific environmental conditions (Guo et al. 2010). Alterations in plant height should also prove useful for improving wheat yield in specific genetic backgrounds, environments, or farming systems (Law et al. 1978; Flintham et al. 1997; Addisu et al. 2010). In addition to *Rht-B1b* and *Rht-D1b*, the GA insensitive (GAI) alleles *Rht-B1c*, *Rht-B1d*, *Rht-B1e*, *Rht-D1c*, and *Rht-D1d* also have an affect on plant height (Borner et al. 1996). The *Rht-1* open reading frames of these alleles have been sequenced and polymorphisms have been identified that extensively or partially explain the associated changes in plant height (Pearce et al. 2011; Wu et al. 2011; Li et al. 2012a, b). The greatest reductions in height and GA sensitivity are associated with *Rht-B1c* and *Rht-D1c* (Izumi et al. 1981; Gale and Youssefian 1985; Borner and Mettin 1988). *Rht-B1e* and *Rht-D1d* are associated with heights slightly shorter than *Rht-B1b* or *Rht-D1b* (Worland and Sayers 1995; Borner et al. 1996) and *Rht-B1d* is associated with heights slightly taller than *Rht-B1b* or *Rht-D1b* (Worland and Petrovic 1988). *Rht-B1b* and *Rht-D1b* are estimated to be in approximately 90 % of the world's semi-dwarf varieties (Worland et al. 1998b); hence the use of other GAI alleles is restricted, although there may be regional use, such as *Rht-B1d* in Italy and southern Europe (Worland and Petrovic 1988). Two additional *Rht-B1* alleles that potentially affect plant height and GA sensitivity were recently identified by Wilhelm et al. (2013b). Both alleles contain a large 5′ insertion (160 or 197 bp) within 600 bp of the *Rht-B1* start codon and, therefore, may disrupt the promoter region and affect DELLA expression. The 160 bp insertion is of particular interest because it occurs in the middle of a 120 bp stretch of sequence that is highly conserved in all three wheat *Rht-1* homoeologs and in *Rht-1 Poaceae* orthologs (Duan et al. 2012; Wilhelm et al. 2013a).

In this study, the frequencies of *Rht-B1a*, *Rht-B1b*, the *Rht-B1* 160 and 197 bp insertions, *Rht-D1a*, *Rht-D1b*, *Ppd-D1a*, and *Ppd-D1b* were determined in a worldwide core collection of 372 wheat varieties (372CC; Balfourier et al. 2007) using PCR markers. Plant height, GA sensitivity, and days to heading (relative to 1 January) were recorded for each accession and correlated with the *Rht-1* and *Ppd-D1* genotype scores and the effect of each allele on the selected plant traits is estimated. In addition, two statistical methods for classifying GA response were validated in relation to *Rht-1* genotype (*Rht-1a* versus *Rht-1b*) of the 372CC

accessions and then used to search for novel GA insensitive alleles in the collection.

Materials and methods

Outdoor experiment design

Plant height and flowering date of the 368 bread wheat accessions present in the 372CC were examined in an outdoor experiment at the National Institute of Agricultural Botany (NIAB), Cambridge, UK in which plants were grown in 4-l pots in outdoor beds. The 372CC is representative of 98 % of the genetic diversity (based on 38 simple sequence repeats) of 3,942 accessions originating from 73 nations and includes landraces and genetically fixed lines with registration years spanning 1830–1998 (Balfourier et al. 2007). Accessions were sown in one of two sowing dates: 25 November 2008 or 18 February 2009. Each pot (experimental unit) contained four plants of an accession along with coarse compost mixed with approximately 10 g of 11-11-18 (N %-P %-K %) controlled-release fertilizer.

The November sowing comprised 249 372CC accessions (primarily winter habit types) along with 16 control lines (Online Resource 1). Controls were winter or facultative habit types. *Ppd-D1* controls included *Ppd-D1a* and *Ppd-D1b* lines. Among the *Rht-1* controls were *Rht-B1a*, *Rht-B1b*, *Rht-B1c*, *Rht-B1d*, *Rht-B1e*, *Rht-D1a*, *Rht-D1b*, and *Rht-D1c* near isogenic lines (NILs) in the 'Cappelle Desprez' and/or 'Mercia' background. Seeds were germinated in compost in 96-well trays in the glasshouse under natural lighting (21 °C for 16 h; 17 °C for 8 h). Following emergence, seedlings were exposed to ambient air temperatures and natural lighting in the glasshouse for 4 weeks before being transplanted to 4-l pots and placed in outdoor beds. The experimental design was an incomplete block composed of two main blocks each consisting of three beds. A pot of each control was placed in each outdoor bed (six replicates per control) and the 372CC accessions were represented once in each main block (two replicates per accession). Experimental units were randomised to achieve maximum efficiency using: http://www.niab.com/dew/.

The February sowing consisted of 119 accessions (primarily spring habit types) along with 13 controls (Online Resource 1). *Ppd-D1* controls included accessions containing *Ppd-D1a* or *Ppd-D1b*. *Rht-1* controls included *Rht-B1a*, *Rht-B1b*, *Rht-B1c*, *Rht-D1a*, and *Rht-D1b* NILs in the genetic backgrounds of the spring lines 'April Bearded' and 'Bersee'. Seeds were sown directly into pots and immediately positioned in the outdoor beds. The February sowing contained two main blocks, each consisting of 1.5 outdoor beds (one outdoor bed was split between two main blocks). A pot of each control was present in each outdoor bed (three replicates per control) and the 372CC accessions were represented once in each main block (two replicates per accession). Experimental units were randomized using the Microsoft Excel random number function.

In both sowings, outdoor beds contained peat and were irrigated as needed. Roots were allowed to grow into the outdoor beds through openings in the bottoms of pots. Each outdoor bed contained 161 4-l pots (7 pots wide × 23 pots long), which included 105 experimental units and 56 pots of cv. 'Xi19' placed along the perimeter to reduce edge effects. Twine was strung across outdoor beds to keep plants upright. To control powdery mildew, fungicides were applied that did not contain known growth regulators. Plants were sprayed to control aphids and covered with netting just prior to harvest to prevent bird damage. Mature plant height was measured as the distance from the soil surface to the tip of the terminal grain of the head (excluding awns) of the longest tiller present in each pot. Days to heading was recorded as the number of days from 1 January until the inflorescence of the primary tiller from each pot was 50 % emerged from the flag leaf.

DNA extraction and genotyping

Leaf tissue was collected from 2- to 3-week-old plants from the outdoor experiment and DNA extracted using a modification of the method described by Fulton et al. (1995). To minimize genotyping errors, genomic DNA (gDNA) was extracted from two replicates of four bulked plants of genetically fixed lines and from four individual plants of landraces, and assayed in separate PCR reactions. PCR assays for *Rht-B1a, Rht-B1b, Rht-D1a, Rht-D1b*, and the presence of the *Rht-B1* 160 bp and 197 bp insertions were performed as described by Wilhelm et al. (2013b). The PCR assay for detecting *Ppd-D1a* was performed as described in Beales et al. (2007).

GA sensitivity experiments

The three experiments to examine GA responsiveness of the 372CC accessions were conducted in the glasshouse at the John Innes Centre (JIC), Norwich, UK. GA experiment 1 (GA expt 1) contained all 368 bread wheat accessions from the 372CC. GA expt 2 contained 161 bread wheat accessions. GA expt 3 contained 19 bread wheat accessions. The successive experiments were required to resolve indeterminate classifications of GA responsiveness from the preceding experiment. Each GA sensitivity experiment contained the following NIL controls: 'April Bearded', 'April Bearded *Rht-D1b*', 'April Bearded *Rht-B1c*', 'Bersee', 'Bersee *Rht-B1b*', 'Mercia', and 'Mercia *Rht-D1b*'.

GA sensitivity tests were based on those described by Gale and Gregory (1977), except a GA− treatment was

included along with the GA+ treatment. The GA+ treatment contained 10 ppm GA_3 (Sigma product number G-7645) dissolved in 0.1 % ethanol. The GA− treatment consisted only of 0.1 % ethanol. For each experiment, an incomplete block design was used with GA treatments randomly assigned to whole trays within main blocks. Trays consisted of 60 wells filled with a peat/sand mixture moistened with a GA+ or GA− solution and each well contained one seedling (the experimental unit). Seedlings were grown from seeds placed on moistened filter paper and stratified at 4 °C for a minimum of 2 days followed by 3 days at room temperature prior to transplanting. Seedlings were randomly assigned a tray and well located within each GA treatment using the Microsoft Excel random number function.

GA expt 1 consisted of three main blocks with seven GA+ trays and seven GA− trays per main block (42 trays total). GA expt 2 consisted of three main blocks with three GA+ trays and three GA− trays per main block (18 trays total). GA expts 1 and 2, therefore, each contained three GA+ and three GA− plants per 372CC entry. GA expt 3 consisted of six main blocks per treatment and a total of two GA+ trays and two GA− trays. Each 372CC entry in GA expt 3 was represented once in each main block (six GA+ and six GA− plants per accession) with the following exceptions that had low germination rates: INRA-03752, two plants per treatment; INRA-00347 and INRA-01065, four plants per treatment. For all three experiments, controls were represented once per tray. Trays were placed in the glasshouse under artificial lighting (300 to 400 μmol m^{-2} s^{-1}) with day/night temperatures of 20 °C/15 °C, 16 h day length. Treatments were applied to trays by gently watering with a GA+ or GA− solution as plants needed moisture. Approximately 3 weeks after each experiment began, plants were uprooted and the length from the seed to first ligule (STFL) was recorded.

Statistical analyses

To classify the GA sensitivity of accessions, a probability distribution mixture (PDM) model and a least significant difference (LSD) test were used. In the PDM model, a mixture model consisting of two independent normal distributions with different means and variances (George et al. 2000) was fitted with a probability (α) of an observation belonging to one distribution and a probability ($1-\alpha$) belonging to a second distribution. An observation consisted of the mean STFL length of an accession in the GA+ treatment in an experiment. All five parameters ($\mu_1, \sigma_1^2, \mu_2, \sigma_2^2$, and α) were fitted by maximum likelihood in Microsoft Excel using the Solver function. Posterior probabilities of any individual observation belonging to either of the two distributions were calculated from the probability density functions of each using the estimated means and variances as $(p1\alpha)(p1\alpha + p2(1-\alpha))^{-1}$ and $(p2\alpha)(p1\alpha + p2(1-\alpha))^{-1}$. A threshold posterior probability greater than or equal to 0.95 was used to assign individual observations to one or the other of the following GA classes: GAI or GA sensitive (GAS). Individuals with posterior probabilities <0.95 for either of the two distributions were classified as having an intermediate (Int) GA response. GA sensitivity classifications based on the PDM model are reported for the GA+ treatment of GA expts 1 and 2, but not for the GA+ treatment of expt 3 or the GA treatment differences (GA trt diff; the STFL length of the GA+ treatment minus the GA− treatment of an accession) of any GA expt because it was not possible to separate these distributions. In the LSD test, the GA trt diff for each accession was tested for statistical significance by *t* test in GA expts 1, 2, and 3. Positive differences significant at the 5 % level were classified as GAS. Variances for the GA+ and GA− treatments were treated as unequal and estimated separately for each experiment, but pooled over all accessions. Variances and standard errors were calculated with Genstat, 12th edition (VSN International). T value probabilities were calculated with the Microsoft Excel TDIST function (two-tailed).

To estimate the effects of *Rht-1* and *Ppd-D1* on the measured plant traits, three sets of restricted maximum likelihood mixed model (REMLMM-1; -2; -3) analyses were conducted using a subset of 352 accessions from the 372CC. This subset excluded accessions containing heterozygotes or rare *Ppd-D1* or *Rht-1* alleles. In the REMLMM-1 analyses for plant height and days to heading, fixed effects were growth habit, *Rht-B1*, *Rht-D1*, *Ppd-D1*, and all 2-way and 3-way interaction terms (Online Resource 2a-b). For REMLMM-1 GA trt diff, fixed effects were *Rht-B1*, *Rht-D1*, *Ppd-D1*, and all interactions (Online Resource 2c). Fixed effects were declared significant at an F probability threshold of 0.05. REMLMM-2 analyses were conducted to estimate mean allelic effects using loci that had significant main effects in the REMLMM-1 analyses in each model. Fixed effects in the REMLMM-2 analyses were as follows: Days to heading = *Rht-B1*, *Ppd-D1*, and *Rht-B1*Ppd-D1*; Plant height = *Rht-B1*, *Rht-D1*, *Ppd-D1*, and all 2-way interactions; GA trt diff = *Rht-B1*, *Rht-D1*, and *Rht-B1*Rht-D1*. REMLMM-3 analyses were performed to estimate the effect of accession type or time period of varietal registration on each plant trait. Year of registration was available for only 192 of the accessions. Fixed effects in the REMLMM-3 analyses (which included significant main effects from the REMLMM-1 analyses) were as follows: Days to heading = growth habit, *Ppd-D1*, *Rht-B1*, all 2-way interactions, and accession type or registration period; Height = *Rht-B1, Rht-D1, Ppd-D1,* all 2-way interactions, and accession type or registration period; GA trt diff = *Rht-B1*, *Rht-D1*, *Rht-B1*Rht-D1*, and

accession type or registration period. Variety served as the random effect in all REMLMM analyses. Fitted and predicted means were compared using an LSD test with a threshold probability of 0.05. REMLMM analyses and comparison of means were performed using Genstat, 12th edition.

Pearson's correlation coefficient (r) was determined using the PEARSON function in Microsoft Excel.

Results

Rht-1 and *Ppd-D1* allelic frequencies

Alleles present at the *Rht-B1*, *Rht-D1*, and *Ppd-D1* loci were determined for each of the 368 bread wheat accessions from the 372CC and in a set of control lines (Online Resource 1). Eight accessions were not homozygous at all three loci and are not included in the summary (Table 1). Among the remaining 360 accessions, the *Rht-B1* 160 bp insertion was present in 58 (16.1 %) of the accessions and the *Rht-B1* 197 bp insertion was present in 43 (11.9 %) of the accessions. The *Rht-B1* insertion data were then correlated with the *Rht-B1a/b* data and with a single exception, the *Rht-B1* insertions occurred in accessions containing the *Rht-B1a* allele. Herein, *Rht-B1a*-0, *Rht-B1a*-160 and *Rht-B1a*-197 will be used to refer to *Rht-B1a* genotypes with no insertion, a 160 bp insertion, and a 197 bp insertion, respectively. The single exception was INRA-06986 (cv. 'Tom Thumb'), for which the product associated with the 197 bp insertion was amplified, but no product was amplified with the *Rht-B1a* or *Rht-B1b* primer pairs even under polymerase extension times of 3 min. 'Tom Thumb' carries the dwarf allele *Rht-B1c,* which contains an insertion of up to 2,026 bp in the region amplified by the *Rht-B1a* and *Rht-B1b* primer pairs (Wu et al. 2011), which likely explains the absence of product in this line and in the four control NILs containing *Rht-B1c*. *Rht–B1a*-0 was the most frequent *Rht-B1* allele occurring in 213 (59.2 %) of the accessions. *Rht-B1b* was present in 45 (12.5 %) of the accessions. Geographically, *Rht-B1a*-160 is most prevalent in accessions originating in Europe, central Russia, and North America, but is also present in low frequency among accessions from Asia and South America (Online Resource 3). The majority (58 %) of the *Rht-B1a*-197 accessions have a French origin, but this allele can also be found in accessions from other European nations, the USA, Australia, Asia, Africa, and South America. *Rht–D1a* and *Rht-D1b* were present in 315 (87.5 %) and 45 (12.5 %) of the homozygous 372CC accessions, respectively.

Four different alleles were discovered with the *Ppd-D1a* PCR assay. The *Ppd-D1b* allele was present in 263 (73.1 %) of the accessions and the insensitive *Ppd-D1a* allele was present in 90 (25.0 %) of the accessions. Accessions containing *Ppd-D1a* were present in most of the geographical regions represented in the collection, although no accessions originating in Northern European nations contained this allele. A third *Ppd-D1* allele was amplified using gDNA of INRA-13812 (D genome derived from *Aegilops tauschii*) in the *Ppd-D1a* PCR assay. The amplified product was approximately 450 bp in length, which is larger than the *Ppd-D1b* (414 bp) or *Ppd-D1a* (297 bp) product. For six of the accessions, no product was

Table 1 Summary of 372CC genotypes

Rht-D1 allele	*Rht-B1* allele[b]	*Ppd-D1* allele (no. of accessions)[a]				Sum[c]
		Ppd-D1a	*Ppd-D1b*	450 bp product	No product	
Rht-D1a	*Rht-B1a*-0	26	161	0	5	192
Rht-D1a	*Rht-B1a*-160	11	43	0	0	54
Rht-D1a	*Rht-B1a*-197	9	22	0	1	32
Rht-D1a	*Rht-B1b*	19	16	1	0	36
Rht-D1a	*Rht-B1c*-197	0	1	0	0	1
Rht-D1b	*Rht-B1a*-0	17	4	0	0	21
Rht-D1b	*Rht-B1a*-160	0	4	0	0	4
Rht-D1b	*Rht-B1a*-197	2	9	0	0	11
Rht-D1b	*Rht-B1b*	6	3	0	0	9
Rht-D1b	*Rht-B1c*	0	0	0	0	0
	Sum	90	263	1	6	360

[a] *Ppd-D1* allelic designations are based on the *Ppd-D1* PCR assay (Beales et al. 2007). The size of the 450 bp PCR product is approximate

[b] −0, −160, and −197 refer to alleles containing no insertion, a 160 bp insertion, or a 197 bp insertion, respectively

[c] A total of 368 accessions were assayed (two to four biological replicates each). Eight accessions were not homozygous in all replicates and were excluded

Table 2 Mean effects of *Rht-1* and *Ppd-D1* alleles on 372CC plant traits

Allele	Mean days to heading[a]			Mean (predicted) plant height (cm)[b]					Mean GA trt diff (mm)[c]		
	Ppd-D1a	*Ppd-D1b*	Fitted mean[d]	*Rht-D1a*	*Rht-D1b*	*Ppd-D1a*	*Ppd-D1b*	Fitted mean	*Rht-D1a*	*Rht-D1b*	Fitted mean
Rht-B1a-160	137 (11)	145.4 (47)	141.2	129.5 (54)	72.7 (4)	82.2 (11)	120 (47)	101.1	28.3 (54)	0.6 (4)	14.4
Rht-B1a-197	136.5 (11)	143.7 (31)	140.1	123.2 (31)	93.2 (11)	103 (11)	113.4 (31)	108.2	27.4 (31)	5.3 (11)	16.3
Rht-B1a-0	134.8 (43)	141.7 (165)	138.3	138.6 (187)	108 (21)	113.7 (43)	132.9 (165)	123.3	29.4 (187)	5.1 (21)	17.3
Rht-B1b	134.6 (25)	142.2 (19)	138.4	101 (35)	71.6 (9)	85.6 (25)	87 (19)	86.3	9.4 (35)	0.7 (9)	5.0
Fitted mean	135.7	143.3		123.1	86.4	96.1	113.3		23.6	2.9	
Fitted means differ at $p \leq 0.05$[e]	*Rht-B1a*-0 < *Rht-B1a*-160; *Ppd-D1a* < *Ppd-D1b*			*Rht-B1b* < *Rht-B1a*-0, *Rht-B1a*-160, *Rht-B1a*-197; *Rht-B1a*-160, *Rht-B1a*-197 < *Rht-B1a*-0; *Rht-D1b* < *Rht-D1a;* *Ppd-D1a* < *Ppd-D1b*					*Rht-B1b* < *Rht-B1a*-0; *Rht-B1b* < *Rht-B1a*-160; *Rht-B1b* < *Rht-B1a*-197; *Rht-D1b* < *Rht-D1a*		

[a] Days from January 1. *Rht-B1a*-160, *Rht-B1a*-197, and *Rht-B1a*-0 refer to *Rht-B1a* alleles with a 160 bp, a 197 bp, or no insertion, respectively. The number of accessions possessing each genotypic combination are indicated in parentheses

[b] Predicted plant height means were derived from a REML mixed model with fixed effects of *Rht-D1*, *Rht-B1, Ppd-D1*, and all two-way interactions. Variety served as the random effect

[c] Mean GA treatment difference (trt diff) refers to the seed to first ligule (STFL) length of the GA+ treatment minus the GA− treatment

[d] Fitted mean refers to the mean effect associated with an allele across genotypes. *Rht-B1* plant height fitted means were identical for *Rht-D1* and *Ppd-D1*

[e] Significant differences are based on a probability (p) threshold of 0.05. Direction of significance is shown by "<"

amplified when multiple biological samples served as template in the *Ppd-D1a* assay, indicating the presence of a fourth *Ppd-D1* allele. The accessions consisted of three landraces from nations near the Black Sea (Azerbaijan, Georgia, and Turkey) and three genetically fixed lines from Russia, Ethiopia, and France.

Plant traits

Among the 372CC bread wheat accessions, days to heading ranged from 116 to 185 days with a mean of 141 days (Online Resource 4a). Plant heights ranged from 54 to 200.5 cm with a mean of 133 cm (Online Resource 4b). For the three GA expts combined, GA trt diff of individual accessions ranged from −14.7 to 54.3 mm with a mean of 23.7 mm (Online Resource 4c). The highest correlation coefficient (r) among traits was between plant height and GA trt diff ($r = 0.58$). The days to heading and GA trt diff r value was 0.10 and the days to heading and plant height r value was 0.18.

Rht-1 and *Ppd-D1* allelic effects on plant traits

For days to heading, *Ppd-D1* accounted for the largest proportion of variation in the REMLMM-1 analysis (Online Resource 2a). The main effects of *Rht-B1* and growth habit were also statistically significant whereas *Rht-D1* and all interactions were not significant. In the REMLMM-2 analysis, *Ppd-D1a* accessions had fitted means (FMs) that were 7.6 days earlier than *Ppd-D1b* accessions, a statistically significant difference at $p \leq 0.05$ (Table 2). The days to heading FM of *Rht-B1a*-0 was also significantly less than that of *Rht-B1a*-160. The six accessions that did not amplify a PCR product with the *Ppd–D1a* primers had a mean days to heading of 142.2 days. INRA-13812, which amplified a 450 bp product with the *Ppd-D1a* primers, had a days to heading of 142 days.

In the plant height REMLMM-1 analysis, the main effects of *Rht-B1* and *Rht-D1* were the greatest and statistically significant (Online Resource 2b). *Ppd-D1* had the next largest effect and was statistically significant, along with six interactions that had smaller effects. In the REMLMM-2 analysis, *Rht-B1b* was associated with statistically significant FM height reductions of 14.8–37.0 cm relative to the other *Rht-B1* alleles (Table 2). Relative to the *Rht-B1a*-0 allele, *Rht-B1a*-160 and *Rht-B1a*-197 both had statistically significant reductions in height of 22.2 and 15.1 cm, respectively, when comparing FMs. *Rht-D1b* accessions had a significant reduction in FM height of 36.7 cm relative to *Rht-D1a* accessions and *Ppd-D1a* accessions had a significant reduction in FM height of 17.2 cm relative to *Ppd-D1b* accessions.

For GA trt diff, the main effects of *Rht-B1* and *Rht-D1* were by far the greatest and the effect of *Rht-B1***Rht-D1* was also significant in the REMLMM-1 analysis (Online Resource 2c). In the REMLMM-2 analysis of GA trt diff, the effect of *Rht-B1b* was statistically significant relative to

all three *Rht-B1a* alleles (9.4–12.3 mm FM reductions) and the FM of *Rht-D1b* accessions was significantly reduced (20.7 mm) relative to *Rht-D1a* accessions (Table 2). The GA trt diff FMs of *Rht-B1a*-160 and *Rht-B1a*-197 accessions were slightly reduced (2.9 and 1.0 mm, respectively) relative to the FM of *Rht-B1a*-0 accessions, but were not statistically significant.

In the REMLMM-3 analysis, the main effects of varietal type and registration period were statistically significant for plant height, but not for days to heading or GA trt diff (Table 3). The predicted mean for plant height in genetically fixed lines was reduced by 14.1 cm relative to landraces (Table 3a). Accessions registered in the three 20-year periods that span 1940–1999 all had significant reductions in height relative to the three time periods that span the years 1800–1939, with differences in predicted means ranging from to 17.3–27.5 cm among registration periods (Table 3b).

GA sensitivity test validation and mutant screen

GA response classifications of the 372CC accessions based on the PDM model and LSD test were validated relative to the *Rht-B1a/b and Rht-D1a/b* genotype scores (Table 4) and then used to search for GAI mutants not containing *Rht-B1b* or *Rht-D1b*. The nine accessions with an *Rht-B1b* + *Rht-D1b* genotype were all classified as GAI using the PDM model and as GAI/Int using the LSD test. The lone 372CC accession containing the *Rht-B1c* allele (INRA-06986; cv. 'Tom Thumb') and the 'April Bearded *Rht-B1c*' control were also both classified as GAI and GAI/Int using the PDM model and LSD test, respectively (Online Resource 1). Of the 74 accessions containing a single semi-dwarf allele, the PDM model classified 65 as GAI, nine as Int, and none as GAS. Use of the LSD test also resulted in the classification of the majority of these accessions as GAI/Int; however, five accessions were classified as GAS (Table 4). INRA-01647 was the only 372CC accession containing a known semi-dwarfing allele that was not classified as GAI by the PDM model or the LSD test. The control 'Bersee *Rht-B1b*' was also not classified as GAI by the LSD test or PDM model while the remaining GAI controls were correctly classified by both methods. Of the 281 *Rht-B1a* + *Rht-D1a* accessions, the PDM model identified one accession (INRA-03752) as GAI, 14 accessions as Int and the remainder as GAS. The LSD test identified one *Rht-B1a* + *Rht-D1a* accession (INRA-04670) as GAI/Int and the remainder as GAS. The *Rht-B1a* + *Rht-D1a* controls were consistently identified as GAS in each experiment using either model.

Table 3 Predicted mean effects of accession type (a) and registration period (b) on 372CC plant traits

		Predicted means[a]		
	Accessions (no.)	Days to heading[b]	Height (cm)	GA trt diff (mm)[c]
(a) Accession type				
Fixed	322	139.7	104.3	13.2
Landrace	46	141.5	118.4	14.7
Significant differences ($p < 0.05$)				
Height: fixed < landrace				
(b) Registration period of accessions (years)				
1800–1899	9	142	125.0	11.0
1900–1919	10	143.3	127.5	15.9
1920–1939	37	142	122.9	11.4
1940–1959	32	139.1	105.6	12.3
1960–1979	55	138.9	103.5	12.1
1980–1999	49	139.2	100.0	13.1
Significant differences ($p < 0.05$)				
Height: 1940–1959, 1960–1979, 1980–1999 < 1800–1899, 1900–1919, 1920–1939				

[a] Predicted means were derived from REML mixed models with the following fixed effects: Days to heading = growth habit, *Ppd-D1, Rht-B1*, all 2-way interactions, and accession type or registration period; Height = *Rht-B1, Rht-D1, Ppd-D1,* all 2-way interactions, and accession type or registration period; GA trt diff = *Rht-B1, Rht-D1, Rht-B1*Rht-D1,* and accession type or registration period. Variety served as the random effect in each model

[b] Days from January 1

[c] GA treatment difference (trt diff) refers to the change in seed to first ligule (STFL) length of the GA+ treatment minus the GA− treatment

Table 4 GA sensitivity classifications of the INRA 372CC accessions by *Rht* genotype

Rht Genotype[c]	No. of accessions	GA classification (no. of accessions)				
		PDM model[a]			LSD test[b]	
		GAS	GAI	Int	GAS	GAI/Int
B1aD1a	281	266	1[d]	14[e]	280	1[f]
B1bD1a	36	0	28	8[g]	4[h]	32
B1aD1b	38	0	37	1[i]	1[j]	37
B1bD1b	9	0	9	0	0	9
B1cD1a	1	0	1	0	0	1
Segregating	3	1	1	1	1	2
Overall	368	267	77	24	286	82

[a] A probability distribution mixture (PDM) model was used to classify GA sensitivity of accessions based solely on the GA+ treatment in expts 1 and 2. Accessions were classified as GA insensitive (GAI) if *p*GAI was ≥ 0.95, GA sensitive (GAS) if *p*GAI ≤ 0.05; or intermediate (Int) if $0.05 < p\text{GAI} < 0.95$

[b] A least significant difference (LSD) test was used to classify GA sensitivity by calculating the probability that an accession's GA treatment difference (GA+ treatment minus GA- treatment) was equal to zero. Accessions were classified as GAS if the probability of no treatment difference was ≤ 0.05 or as GAI/Int if $p > 0.05$

[c] Segregating refers to accessions segregating at either locus

[d] INRA accession 03752

[e] INRA accessions 00964; 01065; 01417; 03278; 03299; 03617; 03896; 04698; 05219; 06396; 06529; 07085; 13481; 14011

[f] INRA accession 04670

[g] INRA accessions 03050; 03804; 05448; 08197; 08578; 13445; 13811; 13812

[h] INRA accessions 02345; 06027; 08194; 13811

[i] INRA accession 01647

[j] INRA accession 01647

Discussion

Each of the five alleles (*Rht-B1a*-160, *Rht-B1a*-197, *Rht-B1b*, *Rht-D1b*, and *Ppd-D1a*) was present in the 372CC at a frequency of 0.119 or higher. The appreciable percentage of lines containing *Rht-B1a*-160 (16.1 %) or *Rht-B1a*-197 (11.9 %), the wide geographic distribution of these alleles, and their presence in recently released lines indicate these alleles are not geographically isolated or rare in modern bread wheat varieties. The 160 bp insertion was present in a 372CC accession released in 1830 and in four 372CC landraces. However, the insertion was not identified in the bread wheat ancestral lines *T. dicoccoides* or *T. dicoccum* (Wilhelm et al. 2013b), suggesting the insertion is a recent event. The 197 bp insertion was not identified in a 372CC landrace; however, the 197 bases were previously identified in *T. dicoccoides* and *T. dicoccum* and in collinear regions of the bread wheat A and D genomes, indicating the presence of the insertion is the ancestral condition (Wilhelm et al. 2013b). *Rht-B1b* and *Rht-D1b* were each present in 12.5 % of the accessions. In a separate study that examined prevalence of these alleles in 67 wheat varieties from 20 countries, *Rht-B1b* occurred in 58 % of the accessions and *Rht-D1b* in 22 % of the accessions (Tosovic-Maric et al. 2008). The relatively low occurrence of *Rht-B1b* and *Rht-D1b* in the 372CC is likely due to the inclusion of a large number of landraces and accessions that pre-date the widespread use of the *Rht-1* semi-dwarfing alleles, which began in the 1960s. The *Rht-B1a/b* and *Rht-D1a/b* assays are not capable of identifying alternative GAI alleles (Pestsova et al. 2008); hence, these alleles, if present, would not have been identified. However, alternative GAI alleles are generally rare (Worland et al. 1998b) and, therefore, are most likely rare in this collection.

The photoperiod insensitive *Ppd-D1a* allele was present in 25.0 % of the accessions and was found in geographical regions worldwide except for Northern Europe. In a collection of more than 400 European wheat varieties released between 1940 and 2000, the *Ppd-D1a* allele was also rare among Northern European accessions (E. Wilhelm and D. Laurie, JIC, unpublished results). *Ppd-D1a* is most suited to growing seasons that are shorter than those found in Northern Europe (Worland et al. 1998a), which explains its absence in this region. The approximately 450 bp *Ppd-D1* allele present in the synthetic line INRA-13812 is of similar size and origin (*Ae. tauschii*) to a 453 bp *Ppd-D1* allele amplified by Guo et al. (2010), suggesting these alleles likely contain the same polymorphism. The 453 bp allele was shown to contain 24 and 15 bp insertions relative to *Ppd-D1b* (Guo et al. 2010), but the effect on days to heading was not estimated. The remaining *Ppd-D1* allele identified using the *Ppd-D1a/b* assay is characterized by the lack of an amplified product. The occurrence of this allele in three landraces from the Black Sea region indicates this was the likely origin. Further research is required to determine the polymorphism associated with this allele and to estimate its effect on heading date.

For days to heading, the *Ppd-D1* locus had the largest effect, but the effect of *Rht-B1* was also significant. Ear emergence of *Ppd-D1a* plants was an average of 7.6 days earlier than *Ppd-D1b* plants. Similarly, Worland et al. (1988) reported that *Ppd-D1a* generally accelerates ear emergence by 6–8 days in the UK, and Bentley et al. (2013) reported a reduction in heading date of 4–9 days associated with *Ppd-D1a* in UK field trials. The cause of the 2.9 delay in days to heading associated with *Rht-B1a*-160 relative to *Rht-B1a*-0 is unclear and requires further investigation. *Rht-B1b* and *Rht-D1b* were not associated with changes in days to heading, which agrees with previous observations (J. Flintham, JIC, pers. comm.).

Changes in plant height were associated with all three loci that were examined and changes in GA sensitivity

were associated with only *Rht-B1* and *Rht-D1*. Among the alleles, *Rht-B1b* and *Rht-D1b* resulted in the greatest reductions in height and GA sensitivity. Based on fitted means (Table 2), *Rht-D1b* accessions were 70 % the height of accessions containing *Rht-D1a*; *Rht-B1b* accessions were 78 % the height of *Rht-B1a* accessions and 70 % the height of *Rht-B1a*-0 accessions; and *Rht-B1b* + *Rht-D1b* accessions were 55 % the height of *Rht-B1a* + *Rht-D1a* accessions. Flintham et al. (1997), using four NIL series, reported that plant heights of *Rht-B1a* + *Rht-D1b, Rht-B1b* + *Rht-D1a,* and *Rht-B1b* + *Rht-D1b* NILs were 83, 86, and 58 %, respectively, that of *Rht-B1a* + *Rht-D1a* NILs. The larger measured effect of the *Rht-1b* alleles in our study relative to Flintham et al. (1997) is at least partially due to the inclusion of landraces and older varieties in the 372CC. These older accessions appear to contain fewer secondary height-reducing alleles than the modern varieties in which *Rht-B1b* and *Rht-D1b* are found (discussed below) and, therefore, may artificially increase the measured effect of these alleles. *Rht-B1b* and *Rht-D1b* height reductions are attributable to a reduced sensitivity to GA as demonstrated by the large and significant reductions in GA trt diff fitted means (Table 2) and by the classification of nearly all accessions containing *Rht-B1b* or *Rht-D1b* as GAI (Table 4). This was expected, as both alleles are well known to have height reductions associated with reduced GA sensitivity (Gale and Youssefian 1985; Peng et al. 1999). GA trt diff was decreased in *Rht-B1b* + *Rht-D1b* accessions relative to accessions containing a single dwarfing allele. This dosage response was also reported by Yamada (1990) and indicates that varieties containing a single *Rht-1* semi-dwarf allele retain some sensitivity to GA, perhaps owing to the remaining homoeologous wild type copies.

Fitted mean plant heights of *Rht-B1a*-160 and *Rht–B1a*-197 accessions were reduced by 18 and 12 %, respectively, relative to *Rht-B1a*-0 accessions under the conditions used in this study. Small reductions in GA sensitivity of 2.9 and 1.0 mm were also associated with *Rht-B1a*-160 and *Rht-B1a*-197, respectively, relative to *Rht-B1a*-0 accessions, but these differences were not statistically significant. In a separate study that measured transcript abundance in leaf and shoot tissue of 5-day-old seedlings using quantitative real time PCR, small reductions in Rht-B1 transcript abundance were reported in 2 of 2 lines containing *Rht-B1a*-160 and in 3 of 3 lines containing *Rht-B1a*-197 relative to lines with no insertion, but these reductions were not statistically significant with the exception of one line that contained *Rht-B1a*-197 (Wilhelm et al. 2013b). Rht-1 expression patterns can differ with tissue type and developmental stage (Pearce et al. 2011); hence, changes in Rht-B1 expression or GA sensitivity associated with *Rht-B1a*-160 or *Rht-B1a*-197, if present, may be greater after the seedling stage or in other tissue types. Both insertions occur within the 600 bases upstream of the *Rht-B1* start codon and the 160 bp insertion occurs within non-coding sequence that is highly conserved among the *Poaceae* (Duan et al. 2012; Wilhelm et al. 2013a); therefore, either insertion has the potential to disrupt an *Rht-1* regulatory region. *Rht-1* regulatory elements have not yet been identified and the phenotypic effects of polymorphisms occurring outside of the coding region have not been characterized. The measured effects of *Rht-B1a*-160 and *Rht-B1a*-197 on plant height in the 372CC indicate these alleles may be useful for intermediate reductions in height relative to *Rht-B1b* and that this is a region that merits further study. To more clearly estimate the effects of these alleles, NILs containing *Rht-B1a*-160 or *Rht-B1a*-197 are being created.

Accessions containing *Ppd-D1a* have plant heights that are reduced by 15 % relative to *Ppd-D1b* accessions when the fitted mean is calculated over the four *Rht-B1* alleles or over the two *Rht-D1* alleles. *Ppd-D1* had almost no effect on GA trt diff. *Ppd-D1a* has a shared ancestry and close genetic linkage with the GA sensitive semi-dwarf allele *Rht8*, both of which are located on chromosome 2D (Worland et al. 1998b; Gasperini et al. 2012). Gasperini et al. (2012) attributed a 13 % reduction in plant height to the semi-dwarf *Rht8* allele and a 3 % reduction to *Ppd-D1a* in a study that assessed recombinant inbred lines segregating at both loci. Assuming tight linkage exists between the semi-dwarf *Rht8* allele and *Ppd-D1a* in the 372CC accessions examined herein, *Rht8* is likely the primarily cause of the height reduction associated with *Ppd-D1a*.

The analysis of registration year period revealed that predicted mean height of accessions released in the three 20-year periods from 1940 to 1999 was 82 % that of the three periods spanning 1800–1939 even after the effects of *Ppd-D1*, *Rht-B1*, and *Rht-D1* were accounted for in the model. Similarly, the predicted mean height of fixed lines was 88 % that of landraces after accounting for the effects of *Ppd-D1*, *Rht-B1*, and *Rht-D1*. These results suggest the presence of secondary alleles in modern bread wheat that further reduce height relative to landraces and older germplasm. The absence of a significant difference in GA trt diff between the pre-1940 and post-1940 groups suggests that this secondary height reduction does not primarily result from changes in GA sensitivity.

The high correlation between plant height and GA trt diff ($r = 0.58$) in this study is not surprising considering reduced stature of *Rht-1b* accessions is due to reduced GA sensitivity (Gale and Youssefian 1985; Peng et al. 1999). Days to heading had little effect on plant height ($r = 0.18$). Similarly, when the 372CC was grown in France, the correlation between plant height and ear emergence was also low ($r = 0.33$; Bordes et al. 2008). The slight increase in r value when the collection was grown in France may

relate to changes in growth conditions or experimental design. Similar to our results, Dotlacil et al. (2003) estimated an *r* value of 0.19 between days to heading and plant height for European winter wheat accessions grown in the Czech Republic.

In most instances, the PDM model and LSD test correctly classified GA response relative to *Rht-B1b* and *Rht-D1b* genotype scores. In the PDM model, none of the accessions containing a known GAI allele were classified as GAS. The LSD test procedure, however, classified five GAI 372CC accessions and the control 'Bersee *Rht-B1b*' as GAS. The misclassifications were due to the large GA treatment difference present in these accessions, which is likely the result of a GA response from the remaining *Rht-1* homoeologs. Among the 372CC accessions that did not contain a known GAI allele, no novel GAI alleles with an effect as strong as *Rht-B1b* or *Rht-D1b* were identified. However, INRA-03752 was classified as GAI in the PDM model and INRA-04670 was classified as GAI by the LSD test. These two accessions along with fourteen others classified as intermediate by the PDM model may have a weak GA response relative to the *Rht-1b* alleles. There may be other accessions with a minor GA response among the remaining 372CC as well, as a weak GA response in combination with the inherent noise of genetic background may require greater replication than that used herein to positively identify such accessions. In this study, the PDM model was superior to the LSD test in regards to correct identification of GAI alleles and only required a GA+ treatment. The drawback of this method is the need for large sets of accessions that contain a sufficient number of GAI and GAS lines to allow for discrimination between the two distributions. For instance, in GA expt 3, the number of GAI lines was insufficient to allow for effective use of the PDM model. Another potential drawback of the PDM method is that accessions with intrinsically long seedling lengths would not be easily detected without a GA− treatment. However, accessions of this nature were not encountered in the diverse 372CC, suggesting this phenotype is rare in modern hexaploid wheat. The statistical methods developed herein provide researchers two options for classifying GA response, with the PDM model being best suited for larger experiments where two defined distributions are expected.

This study provides an estimate of the effects of several alleles at the *Rht-1* and *Ppd-D1* loci in a worldwide collection under near-optimal growing conditions in the UK. Similar to previous studies, *Rht-B1b* and *Rht-D1b* both had strong effects on plant height and GA sensitivity and *Ppd-D1a* had a strong effect on days to heading. The novel *Rht-B1a*-160 and *Rht-B1a*-197 insertion alleles were associated with statistically significant reductions in plant height of 12 and 18 %, respectively, relative to *Rht-B1a*-0. The height reductions were less than those associated with *Rht-D1b* or *Rht-B1b* and were not clearly related to changes in GA sensitivity. Further characterization of the *Rht-B1* insertion alleles is required, but our results suggest these alleles may be useful for fine adjustments in plant height. In addition, the genetic and phenotypic characterization of the 372CC and the development of statistical methods for determining GA response should aid the discovery of novel GAI alleles and the breeding of varieties with height and flowering time suited to specific environments.

Acknowledgments We thank the JIC horticultural staff for help in conducting the GA sensitivity experiments and thank the NIAB prebreeding team and NIAB horticultural staff for assistance in conducting the outdoor experiment. This work was supported by the NIAB trust with assistance from the Biotechnology and Biological Sciences Research Council. E.P.W. was supported by a NIAB PhD studentship.

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

Ethical standards Experiments comply with the current laws of the United Kingdom.

References

Addisu M, Snape JW, Simmonds JR, Gooding MJ (2010) Effects of reduced height (*Rht*) and photoperiod insensitivity (*Ppd*) alleles on yield of wheat in contrasting production systems. Euphytica 172:169–181

Balfourier F, Roussel V, Strelchenko P, Exbrayat-Vinson F, Sourdille P, Boutet G, Koenig J, Ravel C, Mitrofanova O, Beckert M, Charmet G (2007) A worldwide bread wheat core collection arrayed in a 384-well plate. Theor Appl Gen 114:1265–1275

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). Theor Appl Gen 115:721–733

Bentley AR, Horsnell R, Werner CP, Turner AS, Rose GA, Bedard C, Howell P, Wilhelm EP, Mackay IJ, Howells RM, Greenland A, Laurie DA, Gosman N (2013) Short, natural and extended photoperiod response in BC_2F_4 lines of bread wheat with different *Photoperiod-1* (*Ppd-1*) alleles. J Exp Bot. doi: 10.1093/jxb/ert038

Bordes J, Branlard G, Oury FX, Charmet G, Balfourier F (2008) Agronomic characteristics, grain quality and flour rheology of 372 bread wheats in a worldwide core collection. J Cereal Sci 48:569–579

Borner A, Mettin D (1988) The genetic control of gibberellic acid insensitivity of the wheat variety Ai-Bian 1. In: Miller, TE and Koebner, RMD (eds) Proceedings 7th international wheat genetics symposium, Cambridge, Bath Press, Bath, Avon, pp 489–492

Borner A, Plaschke J, Korzun V, Worland AJ (1996) The relationships between the dwarfing genes of wheat and rye. Euphytica 89:69–75

Dotlacil L, Hermuth J, Stehno Z (2003) Earliness, spike productivity and protein content in European winter wheat landraces and obsolete cultivars. Plant Soil Environ 49:67–74

Duan J, Wu J, Liu Y, Xiao J, Zhao G, Gu Y, Jia J, Kong X (2012) New *cis*-regulatory elements in the *Rht-D1b* locus region of wheat. Funct Integr Genomics 12:489–500

Flintham JE, Borner A, Worland AJ, Gale MD (1997) Optimizing wheat grain yield: effects of *Rht* (gibberellin-insensitive) dwarfing genes. J Agric Sci 128:11–25

Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13:207–209

Gale MD, Gregory RS (1977) A rapid method for early generation selection of dwarf genotypes in wheat. Euphytica 26:733–738

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. In:GE Russell (ed) Progress in plant breeding, vol. 1, pp 1–35

Gasperini D, Greenland A, Hedden P, Dreos R, Harwood W, Griffiths S (2012) Genetic and physiological analysis of *Rht8* in bread wheat: an alternative source of semi-dwarfism with a reduced sensitivity to brassinosteroids. J Exp Bot 63:4419–4436

George AW, Visscher PM, Haley CS (2000) Mapping quantitative trait loci in complex pedigrees: a two-step variance component approach. Genetics 156:2081–2092

Guo Z, Song Y, Zhou R, Ren Z, Jia J (2010) Discovery, evaluation and distribution of haplotypes of the wheat *Ppd-D1* gene. New Phytol 185:841–851

Hedden P (2003) The genes of the green revolution. Trends Genet 19:5–9

Izumi N, Sawada S, Sasakuma T (1981) A dominant gene of dwarfism located on chromosome 4D in *Triticum aestivum* cv. 'Ai-bain 1'. Wheat Inf Serv 53:21–24

Law CN, Snape JW, Worland AJ (1978) The genetical relationship between height and yield in wheat. Heredity 40:133–151

Li Y, Xiao J, Wu J, Duan J, Liu Y, Ye X, Zhang X, Guo X, Gu Y, Zhang L, Jia J, Kong X (2012a) A tandem segmental duplication (TSD) in green revolution gene *Rht-D1b* region underlies plant height variation. New Phytol 196:282–291

Li A, Yang W, Guo X, Liu D, Sun J, Zhang A (2012b) Isolation of a gibberellin-insensitive dwarfing gene, *Rht-B1e*, and development of an allele-specific PCR marker. Mol Breeding 30:1443–1451

Matsushika A, Murakami M, Ito S, Nakamichi N, Yamashino T, Mizuno T (2007) Characterization of Circadian-associated pseudo-response regulators: I. Comparative studies on a series of transgenic lines misexpressing five distinctive *PRR* genes in *Arabidopsis thaliana*. Biosci Biotechnol Biochem 71:527–534

Mizuno T, Nakamichi N (2005) Pseudo-response regulators (PRRs) or true oscillator components (TOCs). Plant Cell Physiol 46:677–685

Pearce S, Saville R, Vaughan SP, Chandler PM, Wilhelm EP, Sparks CA, Al-Kaff N, Korolev A, Boulton MI, Phillips AL, Hedden P, Nicholson P, Thomas SG (2011) Molecular characterisation of *Rht-1* dwarfing genes in hexaploid wheat. Plant Physiol 157:1820–1831

Peng JR, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261

Pestsova EG, Korzun V, Borner A (2008) Validation and utilisation of *Rht* dwarfing gene specific markers. Cereal Res Commun 36:235–246

Tosovic-Maric B, Kobiljski B, Obreht D, Vapa L (2008) Evaluation of wheat *Rht* genes using molecular markers. Genetika 40:31–38

Wilhelm EP, Howells RM, Al-Kaff N, Jia J, Baker C, Leverington-Waite MA, Griffiths S, Greenland AJ, Boulton MI, Powell W (2013a) Genetic characterization and mapping of the *Rht-1* homoeologs and flanking sequences in wheat. Theor Appl Gen 126:1321–1336. doi:10.1007/s00122-013-2055-3

Wilhelm EP, Mackay IJ, Saville RJ, Korolev AV, Balfourier F, Greenland AJ, Boulton MI, Powell W (2013b) Haplotype dictionary for the *Rht-1* loci in wheat. Theor Appl Gen. doi:10.1007/s00122-013-2088-7

Worland AJ, Petrovic S (1988) The gibberellic acid insensitive dwarfing gene from the wheat variety Saitama 27. Euphytica 38:55–63

Worland AJ, Sayers EJ (1995) *Rht1*(*B.dw*), an alternative allelic variant for breeding semi-dwarf wheat varieties. Plant Breeding 114:397–400

Worland AJ, Petrovic S, Law CN (1988) Genetic analysis of chromosome 2D of wheat II. The importance of this chromosome to Yugoslavian varieties. Plant Breed 100:247–259

Worland AJ, Borner A, Korzun V, Li WM, Petrovíc S, Sayers EJ (1998a) The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica 100:385–394

Worland AJ, Korzun V, Roder MS, Ganal MW, Law CN (1998b) Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. Theor Appl Gen 96:1110–1120

Wu J, Kong X, Wan J, Liu X, Zhang X, Guo X, Zhou R, Zhao G, Jing R, Fu X, Jia J (2011) Dominant and pleiotropic effects of a GAI gene in wheat results from a lack of interaction between DELLA and GID1. Plant Physiol 157:2120–2130

Yamada T (1990) Classification of GA response, *Rht* genes and culm length in Japanese varieties and landraces of wheat. Euphytica 50:221–239