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A modern Green Revolution gene for reduced height in wheat

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SUMMARY

Increases in the yield of wheat during the Green Revolution of the late 20th century were achieved through the introduction of *Reduced height* (*Rht*) dwarfing genes. The *Rht-B1* and *Rht-D1* loci ensured short stature by limiting the response to the growth-promoting hormone gibberellin, and are now widespread through international breeding programs. Despite this advantage, interference with the plant's response to gibberellin also triggers adverse effects for a range of important agronomic traits, and consequently modern Green Revolution genes are urgently required. In this study, we revisited the genetic control of wheat height using an association mapping approach and a large panel of 1110 worldwide winter wheat cultivars. This led to the identification of a major *Rht* locus on chromosome 6A, *Rht24*, which substantially reduces plant height alone as well as in combination with *Rht-1b* alleles. Remarkably, behind *Rht-D1*, *Rht24* was the second most important locus for reduced height, explaining 15.0% of the genotypic variance and exerting an allele substitution effect of −8.8 cm. Unlike the two *Rht-1b* alleles, plants carrying *Rht24* remain sensitive to gibberellic acid treatment. *Rht24* appears in breeding programs from all countries of origin investigated, with increased frequency over the last decades, indicating that wheat breeders have actively selected for this locus. Taken together, this study reveals *Rht24* as an important *Rht* gene of commercial relevance in worldwide wheat breeding.

Keywords: wheat, plant height, reduced height, Rht, dwarfing, Rht24, *Fusarium*.

INTRODUCTION

Plant height is an important agronomic trait in wheat (*Triticum aestivum*), as it affects lodging and consequently also quality traits and yield. When combined with the high levels of fertilizer used in modern agriculture, tall plants are extremely susceptible to lodging and the reduction of plant height has therefore been an important goal in breeding programs over several decades (Griffiths *et al.*, 2012). Moreover, the fine-tuning of plant height is also of importance in hybrid wheat breeding, where efficient hybrid seed production requires the male to be taller than the female parent (Boeven *et al.*, 2016a). With the Green Revolution the harvest index of wheat was improved by a substantial reduction in height, thereby increasing the production of grain rather than straw (Hedden, 2003). This was facilitated by the identification of major dwarfing genes, which are widespread nowadays in worldwide breeding programs.

The most widely used of these *Reduced height* (*Rht*) loci are the *Rht-B1* and *Rht-D1* homoeoloci, located on group 4 chromosomes, which encode DELLA proteins that act as repressors of growth but are degraded in the presence of gibberellin (Archard *et al.*, 2006; Wilhelm *et al.*, 2013). The *Rht-1* homoeoloci belong to a group of genes known as gibberellic acid (GA)-insensitive dwarfing genes. At the seedling stage, the height-reducing alleles are insensitive to the exogenous application of gibberellin, and in contrast to the wild-type do not respond with increased growth. Notably, however, the GA insensitivity and reductions in cell size conferred by the *Rht-1* loci not only affect stem elongation, and thus height, but also other GA-dependent developmental processes, for example root elongation (Bai *et al.*, 2013), early seedling vigor required for seedling establishment and growth (Rebetzke *et al.*, 2001), and coleoptile length (Botwright *et al.*, 2001). The latter is essential for cultivation in dry environments, as greater seedling vigor and longer coleoptiles facilitate a deeper sowing and thereby access to soil moisture during germination and crop establishment. Deeper sowing of *Rht-B1b*

and *Rht-D1b* carrying wheat cultivars often results in poor seedling emergence, which like the other pleiotropic effects of the GA-insensitive *Rht-1* loci can lead to reductions in yield (Rebetzke *et al.*, 2007).

The other main group of height-reducing genes are responsive to GA. Possibly the best characterized of these GA-responsive dwarfing genes is *Rht8*, which is located on chromosome 2D, close to the major photoperiod regulator *Ppd-D1*. *Rht8* and the photoperiod-insensitive *Ppd-D1a* allele often occur together, but *Ppd-D1* itself has also been reported to have pleiotropic effects on plant height (Börner *et al.*, 1993). Currently around a dozen additional GA-responsive *Rht* loci are known, most of which have not been intensively studied and their effects consequently need to be validated (McIntosh *et al.*, 2013). Furthermore, quantitative trait loci (QTL) mapping studies have confirmed that plant height is a complex trait, controlled by the few known major *Rht* loci but also by minor QTL (Reif *et al.*, 2011; Griffiths *et al.*, 2012; Würschum *et al.*, 2015a).

We have recently reported an association mapping study for plant height in European winter wheat cultivars that identified *Rht-D1* and *Rht-B1* as major contributors to the genotypic variation for height, explaining 41 and 16%, respectively (Würschum *et al.*, 2015a). Interestingly, another major QTL was identified on chromosome 6A, which explained 11% of the genotypic variance in this panel of cultivars. No major *Rht* locus had been reported on that chromosome in wheat, but more recently *Rht24* was described as a novel *Rht* locus located on chromosome 6A and flanked by the two simple sequence repeat (SSR) markers *Xwmc256* and *Xbarc103* (McIntosh *et al.*, 2017; Tian *et al.*, 2017). A QTL for plant height on chromosome 6A has also been reported by a few QTL mapping studies (e.g. Spielmeyer *et al.*, 2007; Griffiths *et al.*, 2012). Our previous analysis indicated that cultivars without an *Rht-1* semi-dwarfing allele can also possess the desired short stature, and that the novel *Rht* locus on chromosome 6A may be a more widely used height-reducing locus with commercial importance.

The aim of this study was therefore to fine-map and further characterize this *Rht* locus towards more targeted utilization in breeding programs. We performed genome-wide association mapping in a worldwide panel of 1110 winter wheat cultivars, which confirmed the existence of a major plant height QTL on chromosome 6A. We show that this locus is likely to be *Rht24*, and found it to explain 15.0% of the genotypic variance, just slightly less than the strongest QTL *Rht-D1*, with 21.3%. We genetically and physically fine-mapped *Rht24* and identified a high number of closely linked markers, two of which were converted into Kompetitive Allele Specific PCR (KASP) markers to facilitate marker-assisted selection. *Rht24* was found in cultivars from all worldwide geographic origins, and has increased in frequency during recent decades. It has an estimated allele substitution effect of −8.8 cm, and reduces height even in *Rht-B1b* or *Rht-D1b* backgrounds. The GA assays showed that *Rht24* belongs to the group of GA-responsive genes. Taken together, our results revealed that, like the *Rht-1* homoeoloci, *Rht24* is a major height-reducing gene in wheat with worldwide importance.

RESULTS

Plant height in worldwide winter wheat shows substantial temporal and geographic variation

Phenotypic variation of plant height was evaluated in a panel of 1110 winter wheat cultivars grown at four locations over two years. We observed a high heritability of 0.95 and a significant genotypic variance (Table S1). The variance components resulting from interactions of the genotype with location, year or location-by-year were also significant, but were negligible compared with the genotypic variance. The adjusted entry means had an average of 89.3 cm, with a minimum of 36.5 cm and a maximum height of 156.7 cm (Figure 1; Table S1). This large variation mirrors the broad sampling of the cultivars from all over the world, but with a focus on European wheat, as well as from different registration periods. Whereas most of the cultivars were released rather recently, some are older and pre-date the Green Revolution. Analysis of plant height as a function of the year of cultivar release showed that these older cultivars were also the taller ones, and that breeding has since reduced plant height, as illustrated by the clear trend towards shorter plants (Figure 1a). Plant height has subsequently remained rather constant in the last four decades. We also observed clear geographic differences, as the cultivars from the USA were the tallest, with a mean of 111.4 cm, whereas cultivars from China were the shortest on average (Figure 1b). This analysis must, however, be treated with caution because of the different age of the cultivars representing each country of origin. The US lines, for example, are all older than 1990, and many of them pre-date the Green Revolution. In addition, adaptation, especially photoperiod response, may also affect plant height, and thus comparison across geographic origins probably contributed to the comparatively short height of the Chinese lines. Nevertheless, when grouped into cultivars released before or after 1990, we still observed substantial differences among countries for the recently released cultivars. For example, the British cultivars were on average approximately 10 cm shorter than those from Germany. The height of released wheat cultivars certainly depends on the preferences of the farmers and the breeders in the respective country, but the optimal plant height also depends on the agricultural production system, especially the use of fertilizer, which varies between countries. Notably, there are also substantial differences in height

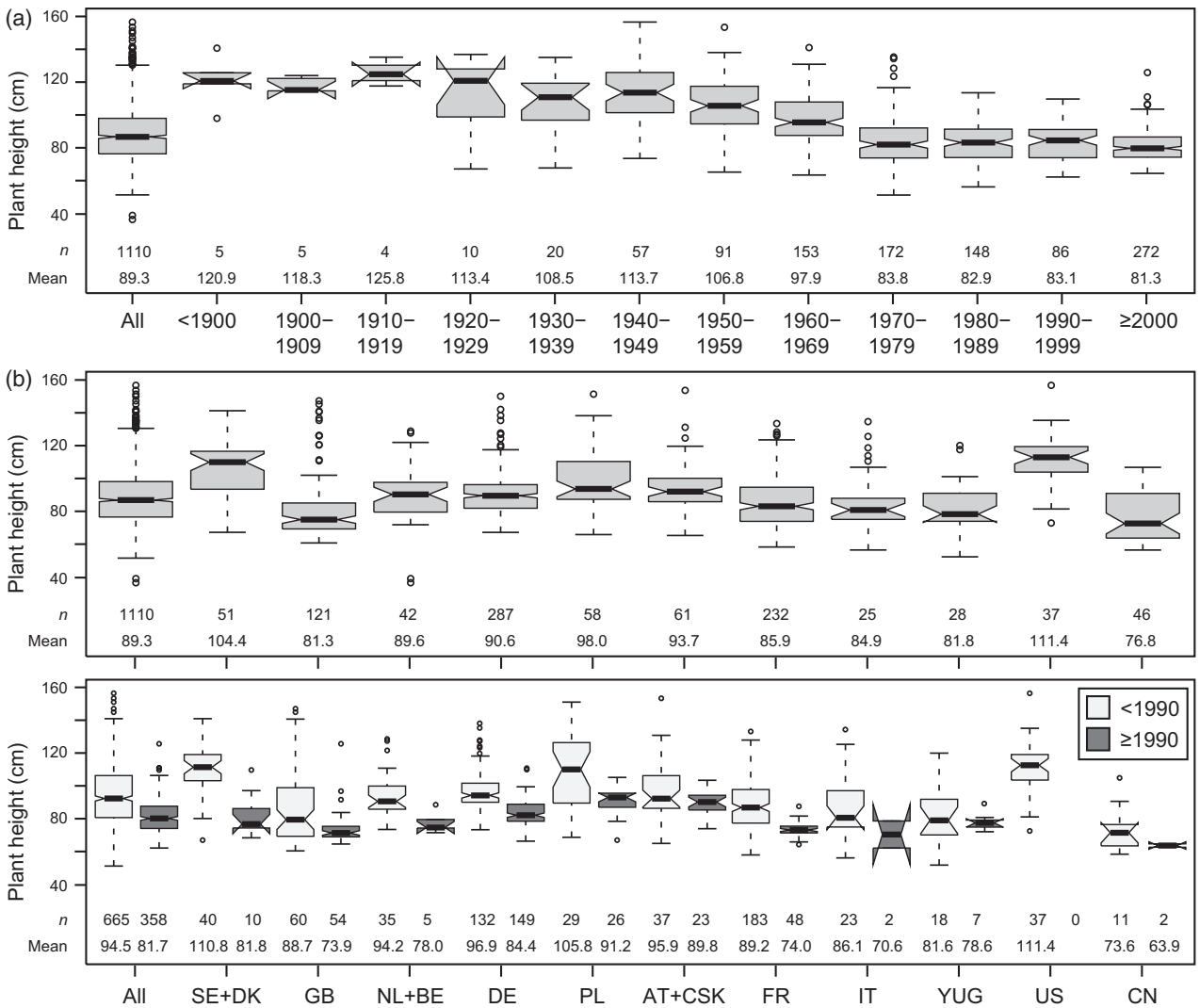

Figure 1. Plant height in cultivars from different decades and origins. (a) Plant height of cultivars from different registration periods and (b) from different countries, as well as for each country from before or after 1990. The year of release was not available for all cultivars. Abbreviations: AT, Austria; BE, Belgium; CN, China; CSK, former Czechoslovakia; DE, Germany; DK, Denmark; FR, France; GB, Great Britain; IT, Italy; NL, the Netherlands; PL, Poland; SE, Sweden; US, United States of America; YUG, former Yugoslavia, Serbia, Croatia.

between cultivars released in the same country, as they originate from different breeders and were bred for different target regions.

The continuous distribution of the trait values illustrates that plant height in wheat is a quantitative trait, suggesting that the variation in height, both within and across countries, is achieved by plant breeding through the differential use of *Reduced height* loci.

The genetic architecture of plant height revealed by genome-wide association mapping

To dissect the genetic architecture underlying the observed variation in plant height in the wheat panel, the cultivars were genotyped by genotyping-by-sequencing, resulting in 44 500 markers, of which 23 720 had a known map position. In addition, all lines were genotyped for *Rht-B1*, *Rht-D1* and *Ppd-D1*, and the most likely chromosomal position of these genes was determined through their linkage disequilibrium (LD) with mapped markers (Figure S1). The genome-wide scan for marker–trait associations resulted in 75 markers significantly associated with plant height, 59 with known map positions, which likely correspond to six QTL (Figure 2; Table 1). These six QTL jointly explained 53.0% of the genotypic variance. It must be noted that the genetic architecture of height in wheat is also likely to include additional genes that remained undetected, either because of the lack of a marker in sufficient LD, because of their small effect size, or because of the low frequency of the height-reducing allele.

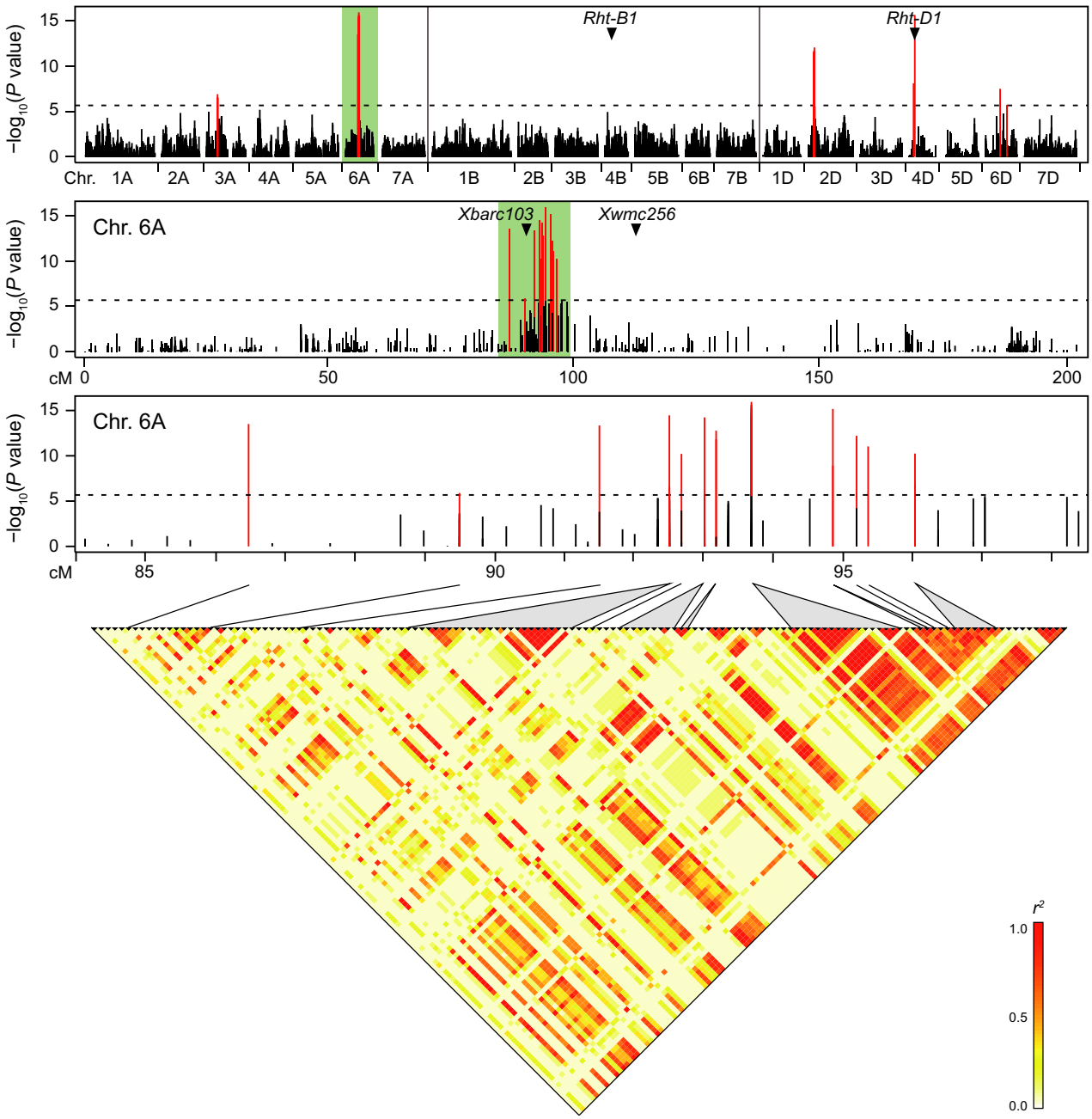

Figure 2. Identification of the *Rht24* locus. Manhattan plot from the genome-wide scan, chromosome 6A, the *Rht24* region on 6A and linkage disequilibrium (r^2) of the markers in that region. [Colour figure can be viewed at wileyonlinelibrary.com].

Rht-D1 on chromosome 4D was identified as a major QTL, explaining 21.3% of the genotypic variance, with an allele substitution effect of −9.4 cm. By contrast, no significantly associated marker was found on chromosome 4B, where *Rht-B1* is located. This may be the result of insufficient marker coverage in the *Rht-B1* region, as the highest LD of *Rht-B1* with any of the mapped markers was rather low, with $r^2 = 0.23$. Nevertheless, when tested directly, *Rht-B1* explained 2.7% of the genotypic variance and had an allele substitution effect of −7.6 cm. This illustrates that in this winter wheat panel *Rht-D1* is more important than its homoeologue *Rht-B1*. Notably, however, the proportion of variance explained by a locus also strongly depends on its allele frequency, which was much lower for *Rht-B1*, probably as this gene is not commonly used in the countries from which the majority of this panel originates. Another peak of significantly associated markers was found on chromosome 2D, which is known to harbor *Ppd-D1* and

Table 1 Quantitative trait loci identified for plant height

	Chr.	Pos. (cM)	*P*	p_G	α-effect[a]	*p*
qRht.3A[b]	3A	75.9	$1.2e^{-7}$	2.3	−2.8	0.73
Rht24[†]	6A	93.7	$1.1e^{-16}$	15.0	−8.8	0.41
Rht-B1	4B	47.8[c]	$1.7e^{-6}$	2.7	−7.6	0.07
qRht.2D[b]	2D	44.5[d]	$3.4e^{-15}$	4.8	3.6	0.26
Ppd-D1	2D	40.8[c]	$2.1e^{-13}$	7.4	−7.8	0.19
Rht-D1	4D	32.8[c]	$3.4e^{-26}$	21.3	−9.4	0.22

p_G, proportion of explained genotypic variance; *p*, frequency of the allele with the height-reducing effect.
[a]Effects of the alleles *Rht24b*, *Rht-B1b*, *Rht-D1b* and *Ppd-D1a*, and for *qRht.3A* and *qRht.2D* 'presence of DArT'.
[b]Data for *Rht24* are shown for the most strongly associated marker S3222505, data for *qRht.3A* are shown for the most strongly associated marker D996159, and data for *qRht.2D* are shown for the most strongly associated marker D1216036.
[c]The most likely position of the candidate genes was determined based on their linkage disequilibrium with mapped markers.
[d]Mapped on chromosome 2A by Li *et al.* (2015), but with a high linkage disequilibrium (r^2) of 0.92 with markers at 44.5 cM on chromosome 2D.

the closely linked GA-sensitive *Rht8* locus. We found that a locus, *qRht.2D*, located a few cM away from *Ppd-D1*, was strongly associated with plant height and explained 4.8% of the genotypic variance. Interestingly, *Ppd-D1* also explained 7.4% of the genotypic variance, even in the presence of *qRht.2D* in the model, and had an allele substitution effect of −7.8 cm. This indicates a pleiotropic effect of *Ppd-D1* on plant height and the presence of another *Rht* locus close by, probably *Rht8*. The two significantly associated markers on chromosome 6D were both in LD with the QTL on chromosome 2D, and did not explain any genotypic variance in the joint fit with *Ppd-D1* and *qRht.2D*. This indicates that they were detected due to their LD with the QTL on 2D, which requires further research. Another minor QTL, explaining 2.3% of the genotypic variance, was identified on chromosome 3A. In addition to this, another major QTL was identified on chromosome 6A. This QTL explained 15.0% of the genotypic variance and had an allele substitution effect of −8.8 cm. As this was the second major QTL besides *Rht-D1*, this locus required further investigation.

The major *Rht* locus on chromosome 6A is *Rht24*

Only one *Rht* locus, *Rht24*, has been described on chromosome 6A of wheat to date. *Rht24* was only recently reported to be located between the two SSR markers *Xbarc103* and *Xwmc256*. To test whether our QTL on chromosome 6A corresponds to *Rht24*, we genotyped a subset of the panel with these two SSR markers and assessed their LD with the mapped markers to place them on our genetic linkage map. We found that *Xbarc103* was in highest LD (r^2 = 0.48) with a marker on chromosome 6A at 91.2 cM, whereas *Xwmc256* showed the highest LD value (r^2 = 0.67) with a marker at 114.6 cM (Figure 2). Thus, the two SSR markers reported to flank *Rht24* also flank our major QTL, strongly indicating that the QTL identified here on chromosome 6A is *Rht24*.

Rht24 was identified by 34 mapped and 11 unmapped markers (Table S2). Based on the map of Li *et al.* (2015), the mapped markers were located between 86 and 96 cM. We therefore analyzed the LD pattern in that chromosomal region and found that even though the significantly associated markers were distributed over 10 cM, they were all in high LD with each other (Figure 2). Interspersed are groups of markers that are not significantly associated with plant height, but which are also not in high LD with the significantly associated markers. We therefore clustered the markers, which revealed that the significantly associated markers form one cluster (Figures S2, S3). This indicates that the examined chromosomal region contains only one *Rht* locus and that the identified significantly associated markers delimit the genomic location of *Rht24*.

To further investigate this *Rht24* genomic region, we aligned the sequences of the markers from this region with the wheat reference genome (IWGSC RefSeq v1.0; Figure 3; Table S2). This revealed that almost all of the significantly associated markers are located in an approximately 50-Mbp region between 400 and 450 Mbp. This genomic region corresponds to the region around 79 cM in the map of Wang *et al.* (2014), based on the wheat 90k single-nucleotide polymorphism (SNP) array. Although the sequence of the SSR marker *Xbarc103* was found at 176 Mbp, *Xwmc256* was again located distal to *Rht24* at 549 Mbp. Thus, the results from the genetic and the physical maps are in good agreement, both supporting the conclusion that the identified locus is *Rht24* and delimiting its chromosomal location for future cloning of the gene.

Interestingly, the GA-sensitive *Rht18* locus from durum wheat has recently been mapped to chromosome 6A, and was also shown to reduce height when introgressed into hexaploid wheat (Yang *et al.*, 2015; Vikhe *et al.*, 2017). We aligned the sequence of the marker reported to co-segregate with *Rht18* with the wheat reference genome and found it to be physically located at 413 Mbp. Thus, *Rht18* appears to be located in the same chromosomal region as *Rht24*. Moreover, Haque *et al.* (2011) found the three GA-sensitive dwarfing genes from durum wheat *Rht14*, *Rht16* and *Rht18* to be linked to the same SSR marker, and additional allelism tests further supported the conclusion that these three *Rht* loci are allelic. Cloning of *Rht24* is required to show whether *Rht14*, *Rht16*, *Rht18*, and *Rht24* are: (i) different genes in the same chromosomal region; (ii) different genes from a cluster of *Rht* genes; or (iii) alleles of the same gene.

Rht24 is used in worldwide wheat breeding

We next assessed the relevance of *Rht24*, the two *Rht-1* homoeoloci, and the other identified *Rht* QTL in worldwide

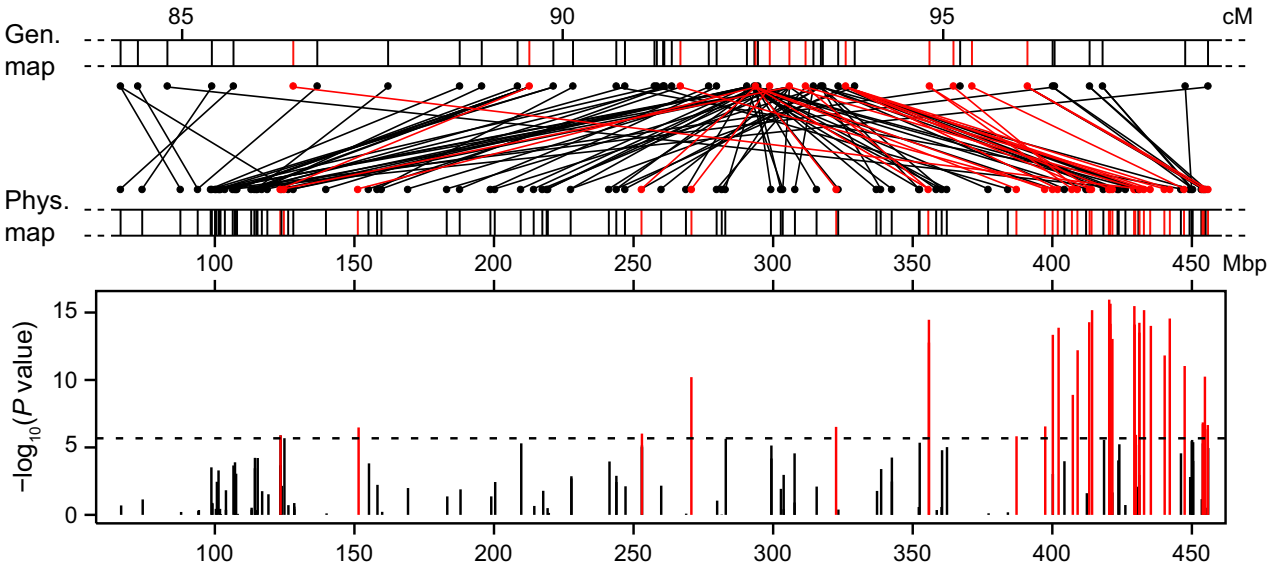

Figure 3. Alignment of the genetic and physical (based on IWGSC RefSeq v1.0) maps for the Rht24 region, with the corresponding Manhattan plot.[Colour figure can be viewed at wileyonlinelibrary.com].

wheat (Table 2). For this analysis we assumed that the most strongly associated marker S3222505 is indicative for *Rht24*, which is supported by our finding that the cultivars generally vary little for the entire haploblock of the 24 most strongly associated markers from the *Rht24* cluster of markers (Figure S2; Table S3). A truly diagnostic marker, however, requires cloning of *Rht24*, and consequently the diagnostic power of the presented markers must be validated in each country and breeding program. Nevertheless, this analysis indicated that *Rht24* is present in wheat cultivars from all countries of origin investigated, including European countries, China and the USA (Table 2). Regarding the three major wheat-producing countries in Europe, the height-reducing *Rht24b* allele had a frequency of 0.37 in German, 0.51 in French and 0.55 in British cultivars. The lowest frequency was observed for the US lines with only 0.08, whereas *Rht24b* showed the highest frequency in the Chinese cultivars with 0.61. The proportion of explained genotypic variance was only 1.8% in the Chinese cultivars, however, indicating either that *Rht24* is of less relevance or that the markers employed are not indicative for *Rht24* in Chinese material. The proportion of genotypic variance explained by *Rht24* varied substantially between countries, and was highest for the Polish lines with 37.2%. For the German and the British cultivars *Rht24* explained 8.5 and 11.3% of the genotypic variance, respectively, and thus approximately one-quarter of the variance explained by *Rht-D1*. For the French cultivars, by contrast, *Rht24* explained 24.5% of the genotypic variance, compared with 19.9% for *Rht-D1*, and also showed a much higher frequency than *Rht-D1*, with 0.51 compared with 0.19 of *Rht-D1*.

We next analyzed the frequency of the height-reducing alleles of *Rht-B1*, *Rht-D1* and *Rht24* over time (Figure 4). This revealed that *Rht-D1b* increased dramatically, beginning in the 1970s, whereas *Rht-B1b* showed a less pronounced increase, probably again because of the composition of the wheat panel. *Rht24b* also showed a strong increase in recent decades, and was found in 67% of the cultivars registered since 1990. By comparison, *Rht-D1b* was found in only 49% of these cultivars. Interestingly, the introgression of *Rht24b* into worldwide wheat appears to have started already before that of *Rht-D1b*, and thus before the Green Revolution. The analysis of the different countries of origin individually revealed that in all cases, *Rht24b* has increased substantially in the cultivars registered after 1990, compared with those registered before. This indicates that *Rht24* has been present in wheat breeding programs for a long time but has recently been increasingly used by wheat breeders worldwide to reduce plant stature to the desired height.

The *Rht-1* homoeoloci and *Rht24* reduce wheat height alone, and in combination

The two *Rht-1* homoeoloci are well known in wheat breeding and are used worldwide, albeit with different preferences in different geographic and climatic regions. We therefore evaluated the potential of *Rht24* to reduce plant height alone, as well as in *Rht-1b* genetic backgrounds. In agreement with the estimated allele substitution effects, we observed a substantial reduction in height for all three *Rht* genes (Figure 5). Interestingly, we found that *Rht24b* reduced height even in plants carrying *Rht-B1b* or *Rht-D1b*. Of the 452 cultivars that carry *Rht24b*, 41 also carry *Rht-*

Table 2 Effect of the *Rht* loci and identified quantitative trait loci, dependent on the country of origin of the cultivar

					qRht.3A			*Rht24*[a]			*Rht-B1*			*qRht.2D*			*Ppd-D1*			*Rht-D1*		
	n	Min	Mean	Max	p_G	α	*p*	p_G	α	*p*	p_G	α	*p*	p_G	α	*p*	p_G	α	*p*	p_G	α	*p*
SE + DK	51	67.1	104.4	141.2	0.0	−2.1	0.73	3.8	−17.7	0.18	–	–	0.00	0.1	−1.7	0.10	–	–	0.00	63.8	−18.6	0.20
GB	121	66.7	81.3	147.2	1.8	−6.2	0.80	11.3	−8.8	0.55	3.7	−5.3	0.07	0.1	−2.5	0.11	0.1	−6.6	0.02	42.6	−12.6	0.56
ND + BL	42	36.5	89.6	128.8	13.9	−7.7	0.86	19.9	−9.3	0.60	–	–	0.00	2.8	−8.5	0.14	2.7	−2.0	0.02	17.2	−7.9	0.10
DE	287	66.9	90.6	150.0	1.2	−0.5	0.80	8.5	−5.9	0.37	1.3	−3.1	0.02	0.1	−0.2	0.18	0.5	−4.9	0.01	32.8	−7.8	0.29
PL	58	65.8	98.0	151.3	5.1	−4.7	0.71	37.2	−11.8	0.43	–	–	0.00	0.3	3.7	0.33	5.6	−9.6	0.21	5.5	−16.4	0.02
AT + CSK	61	65.2	93.7	153.6	14.8	−2.9	0.67	12.3	−6.0	0.25	0.3	−11.7	0.02	4.3	2.1	0.48	5.1	−8.6	0.08	5.5	−6.0	0.08
FR	232	58.2	85.9	133.3	0.1	−0.9	0.82	24.5	−9.3	0.51	0.4	−6.8	0.05	1.1	4.0	0.14	5.2	−7.5	0.22	19.9	−8.3	0.19
IT	25	56.4	84.9	134.5	1.0	−4.4	0.60	11.5	−6.9	0.32	4.6	−12.8	0.16	36.8	14.1	0.44	8.4	−13.3	0.50	8.1	−14.8	0.04
YUG	28	52.0	81.8	119.9	0.0	−0.8	0.75	22.3	−6.6	0.46	16.2	−6.5	0.21	18.5	8.9	0.79	6.9	−11.2	0.82	0.6	−2.9	0.04
US	37	72.7	111.4	156.7	7.0	−7.0	0.43	8.6	−12.6	0.08	–	–	0.00	9.3	6.9	0.11	–	–	0.00	28.4	−18.2	0.05
CN	46	56.4	76.8	106.7	0.1	−1.3	0.50	1.8	−2.4	0.61	15.1	−8.3	0.17	3.7	4.7	0.70	20.6	−10.8	0.80	17.4	−7.9	0.20

p_G, proportion of explained genotypic variance; α, allele substitution effect; *p*, frequency of the allele with the height-reducing effect in the entire panel.
Abbreviations: AT, Austria; BE, Belgium; CN, China; CSK, former Czechoslovakia; DE, Germany; DK, Denmark; FR, France; GB, Great Britain; IT, Italy; NL, the Netherlands; PL, Poland; SE, Sweden; US, United States of America; YUG, former Yugoslavia, Serbia, Croatia.
[a]Data for *Rht24* are shown for the most strongly associated marker S3222505, data for *qRht.3A* are shown for the most strongly associated marker D996159 and data for *qRht.2D* are shown for the most strongly associated marker D1216036.

B1b and 159 carry *Rht-D1b*, whereas the combination of *Rht-B1b* and *Rht-D1b* was rare, found in only three individuals. Thus, *Rht24b* is apparently already employed in combination with the two *Rht-1b* semi-dwarfing genes to further reduce wheat height. To facilitate marker-assisted selection for *Rht24* in breeding programs, markers from the *Rht24* chromosomal region can be converted into functional markers (Table S2). To demonstrate this, we converted two of the SNP markers from the *Rht24* cluster of markers into KASP markers that allow a high-throughput screening for *Rht24* (Figure S4). Taken together, *Rht24* appears to be a valuable *Rht* locus for wheat breeding, either alone or in combination with *Rht-1* loci, and selection can be facilitated by the establishment of functional markers.

Rht24 belongs to the group of GA-responsive dwarfing genes

To determine whether *Rht24* belongs to the group of GA-sensitive or -insensitive genes, we performed GA sensitivity assays. Plants were grown in paper towels at 20°C in either water alone, as a control, or in water supplemented with 50 mg/L GA (GA50). The seedlings were assessed after 10 days, after which the length of the first leaf sheaf, i.e. the length to the first leaf node, was measured (Figure 6). The analysis included five cultivars for wild-type, *Rht-B1b*, *Rht-D1b* and *Rht24b*, which were each assessed in four replicates with 10 plants per replicate and cultivar. As expected, *Rht-B1b* and *Rht-D1b* plants showed no response to GA50 as compared with the control, in line with the GA-insensitivity of these two genes. By contrast, *Rht24b* plants responded to GA and showed a significant elongation of the first leaf sheaf in the GA50 treatment, similar to the wild-type plants. Thus, the *Rht-1* homoeoloci remain the only two GA-insensitive wheat dwarfing genes known to date, as *Rht24* belongs to the group of GA-sensitive genes. Notably, this does not rule out an involvement of GA in the reduced-height phenotype of *Rht24*, but only shows that *Rht24* is not part of the molecular machinery controlling the plant's response to GA. Interestingly, *Rht24b* plants were as tall as the wild-type plants when exogenous GA was applied, but were somewhat shorter in the water control. This indicates that it may be the level of available endogenous GA that restricts height in *Rht24b* plants. Thus, *Rht24* might be involved in GA production or turnover, leading to a GA deficiency, or may result in a more indirect modification of the GA metabolism.

DISCUSSION

Plant height is an important agronomic trait in wheat, and the Green Revolution has moved the *Reduced height* loci to center stage. Of the more than 20 *Rht* genes recorded to date (McIntosh *et al.*, 2013, 2017), most are likely to have low potential for breeding, as they were derived as

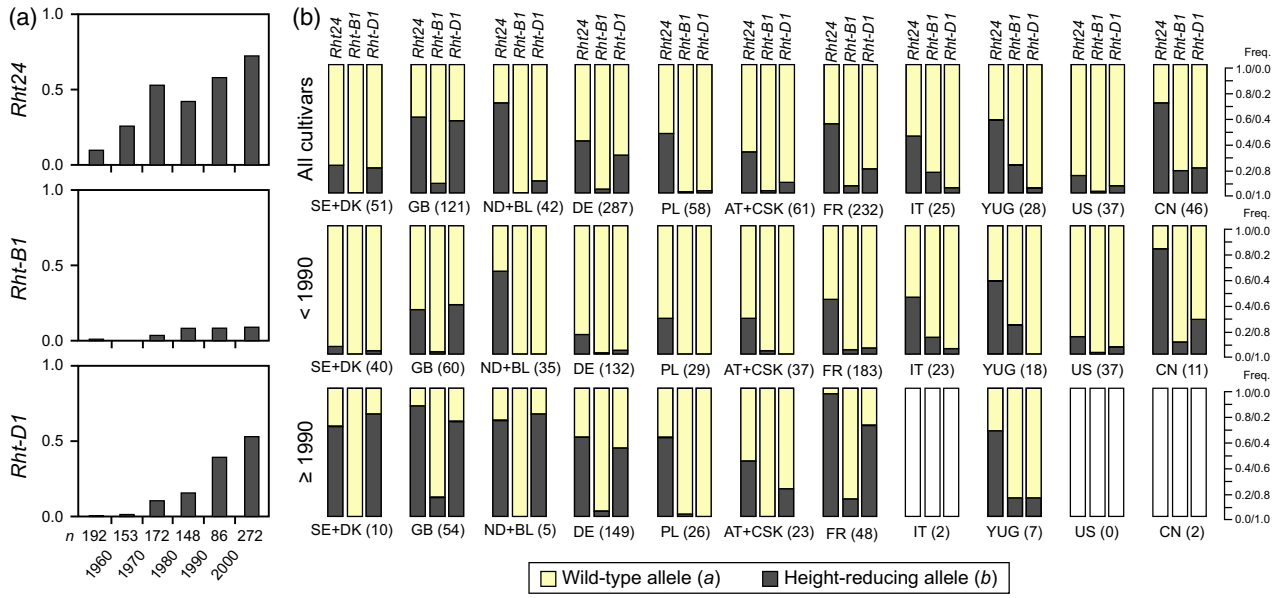

Figure 4. Development of the frequency of the height-reducing allele of *Rht24* (S3222505), *Rht-B1* and *Rht-D1* (a) over time and (b) in all cultivars, as well as those registered before and after 1990, shown for the different countries of origin. Abbreviations: AT, Austria; BE, Belgium; CN, China; CSK, former Czechoslovakia; DE, Germany; DK, Denmark; FR, France; GB, Great Britain; IT, Italy; NL, the Netherlands; PL, Poland; SE, Sweden; US, United States of America; YUG, former Yugoslavia, Serbia, Croatia. [Colour figure can be viewed at wileyonlinelibrary.com].

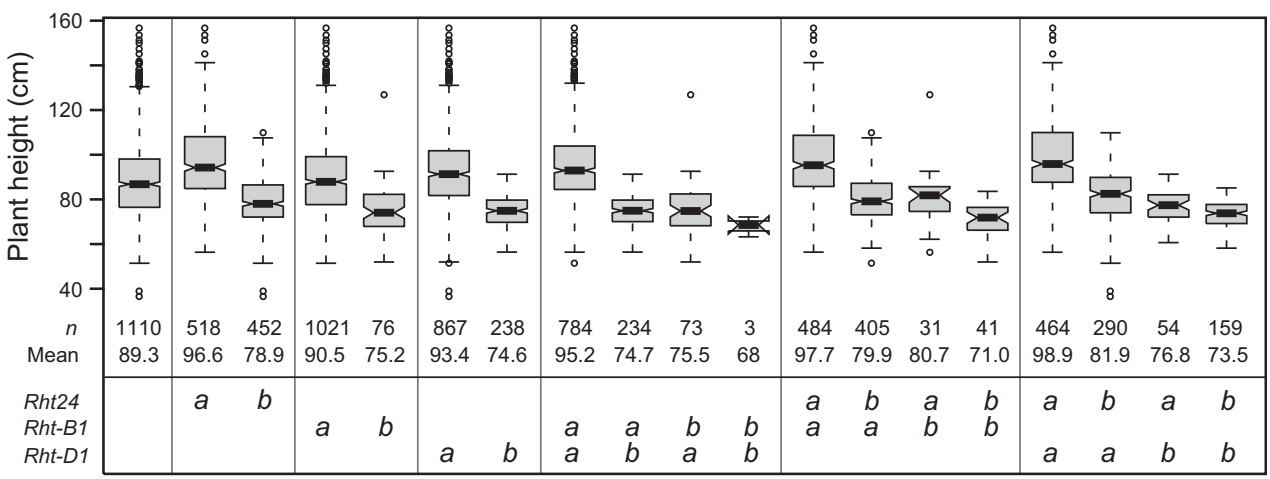

Figure 5. Effects of the *Rht24* (S3222505), *Rht-B1* and *Rht-D1* loci, as well as combinations thereof, on plant height.

mutants. Thus, only a few *Rht* genes that reduce height without adverse effects on yield have been described so far. The most prominent genes are the two *Rht-1* homoeoloci, *Rht-B1* and *Rht-D1*, which have been employed in wheat breeding worldwide since the Green Revolution. In addition to reducing height, they exert a pleiotropic effect and can improve spikelet fertility (Worland and Snape, 2001). Although these semi-dwarfing genes offer the potential of improving the harvest index and thereby yield, a reduction in height generally reduces yield (Law *et al.*, 1973). Notably, the two *Rht-1* semi-dwarfing genes only improve yield under optimal conditions, whereas in lower fertility environments tall isogenic lines without *Rht-B1b* or *Rht-D1b* yielded more than their *Rht-1b*-carrying counterparts (Worland and Snape, 2001). Furthermore, Worland and Law (1985), also using *Rht-1* isogenic lines, demonstrated that these semi-dwarfing genes show large environmental interactions and have a lowered fertility and thus yield when subjected to high temperatures over 24°C prior to ear emergence. As these conditions are regularly met in regions such as southern Europe, height reduction in cultivars bred for these regions appears to be achieved through other loci. Consistently, we found that in cultivars from Italy and the former Yugoslavia, *qRht.2D*, which is

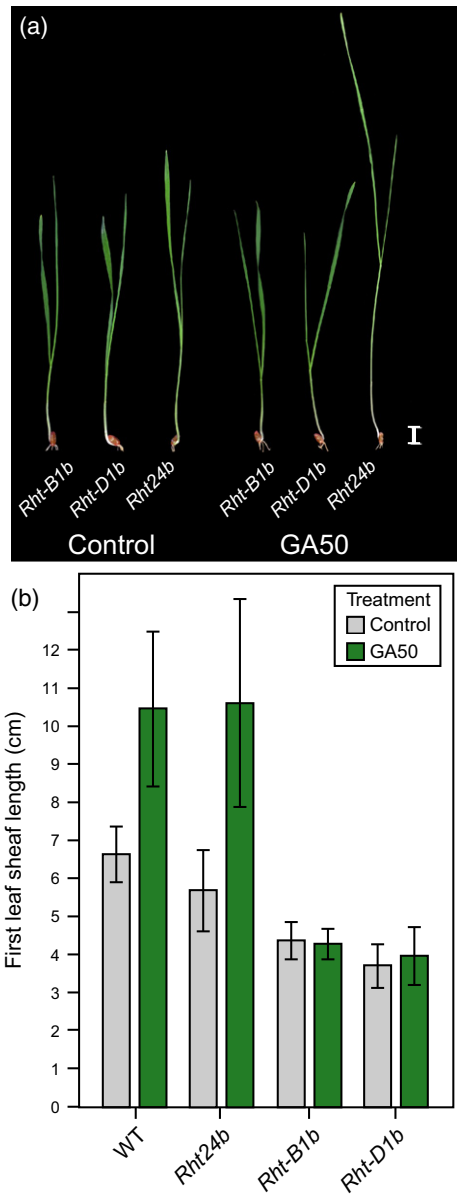

Figure 6. Gibberellic acid sensitivity assay. (a) Response of wild-type (WT), *Rht-B1b*, *Rht-D1b* or *Rht24b* seedlings after 10 days of treatment with control (0 mg/L GA) or with 50 mg/L gibberellic acid (GA50). Scale bar: 1 cm. (b) Length of the first leaf sheaf, i.e. length to the first leaf node, of five cultivars for each *Reduced height* gene, assessed in four replicates with 10 plants. The whiskers represent the standard deviation. [Colour figure can be viewed at wileyonlinelibrary.com].

likely to correspond to *Rht8*, and *Ppd-D1a* were common, whereas *Rht-B1b* and particularly *Rht-D1b* were comparably rare. This illustrates that the choice of *Rht* loci best suited to achieve the desired reduction in plant height must take into account their effects on other agronomic traits and biotic as well as abiotic stresses in the target region. While the gibberellic acid insensitivity of the *Rht-1* loci results in the desired reduction in height, it also ensues a range of pleiotropic, undesired effects. Consequently, a diversification of the *Rht* loci available in wheat breeding is required in order to meet the specific requirements of the diverse target environments.

Interestingly, *Rht24* appears to be used in wheat breeding throughout Europe, without an apparent geographic and thus climatic trend. This may indicate that *Rht24* is less susceptible to environmental variation than the *Rht-1* loci, and may therefore have no or at least less adverse effects on yield under a broad range of climatic conditions. Future work, however, ideally using near-isogenic lines for *Rht24* tested in yield trials under defined climatic conditions, is required to determine the effects of *Rht24* on plant height, agronomic traits and yield dependent on the environment. Nevertheless, the different frequencies across countries may indicate a certain interaction of *Rht24* with the agroclimatic conditions or the production system, result from different preferences for the final height of the wheat cultivars or simply reflect the different times when *Rht24* was introduced into the respective breeding programs.

The *Rht-1* loci were initially brought into worldwide wheat to improve the harvest index. Thus, improvements in grain yield were achieved through shorter plants with more ears and a higher number of grains per ear (Austin *et al.*, 1989). The analysis of winter wheat of different ages grown under uniform conditions revealed a continuous increase in the harvest index from 0.36 to 0.51 for wheat between 1900 and 1980, combined with an increasing grain yield, but with a constant yield of total biomass (Austin *et al.*, 1989; Hay, 1995). It is assumed, however, that the harvest index has a physiological upper limit (Austin, 1980). Thus, when the harvest index of modern cultivars is nearing its upper theoretical limit, further improvements in grain yield can only be achieved by simultaneously increasing biomass yield. This may require adjustments in height towards somewhat taller plants, which would be facilitated by a large portfolio of *Rht* genes and a detailed knowledge of their alleles. Interestingly, almost half of the cultivars evaluated carry the *Rht24* haplotype, whereas only ~21% carry *Rht-D1b* and ~7% carry *Rht-B1b*. As mentioned above, this certainly depends on the composition of the panel, but may also indicate a certain preference of wheat breeders for *Rht24*. Notably, *Rht24* appears to be prevalent in high-yielding wheat regions with high natural disease pressure, such as France and the UK, where it is employed alone or in combination with *Rht-D1b*. Thus, the selection for high-yielding cultivars may have already favored the selection for *Rht24* as compared with the *Rht-1* loci.

Apart from lodging tolerance, harvest index and grain yield, plant height is also associated with *Fusarium* head blight (FHB) resistance (Srinivasachary *et al.*, 2008; Lu *et al.*, 2013). In particular, the two semi-dwarfing genes *Rht-B1b* and *Rht-D1b* were shown to be associated with type I susceptibility to FHB in wheat. This may in part be

related to the effect of the *Rht-1b* alleles in reducing anther extrusion, which in turn has been related to resistance to the initial FHB infection (Buerstmayr and Buerstmayr, 2015; Boeven *et al.*, 2016a; He *et al.*, 2016). A higher proportion of retained anthers may support *Fusarium* colonization of the floral cavity and initial hyphal growth (Buerstmayr and Buerstmary 2016). A recent genome-wide association study did not identify a QTL for anther extrusion on chromosome 6A, indicating that *Rht24* may have no effect on anther extrusion (Boeven *et al.*, 2016a). Interestingly, *Rht8*, which like *Rht24* is also GA sensitive, has been shown to have far less adverse effects on FHB susceptibility than the GA-insensitive *Rht-1* loci (Miedaner and Voss, 2008). Thus, *Rht24* may be an attractive alternative to the *Rht-1* genes to reduce plant height without the negative effects on FHB resistance, and this warrants further research.

Moreover, anther extrusion is an important trait in hybrid wheat breeding, as it determines hybrid seed production and thus the economic success of the hybrid breeding program (Whitford *et al.*, 2013; Boeven *et al.*, 2016a). At the same time, the height of the two parental components must be finely tuned to facilitate maximum cross-pollination. Boeven *et al.* (2016b) have recently described nine lines known to be good males with favorable floral characteristics. Interestingly, all these lines carry *Rht24b* and with only one exception are wild-type for *Rht-B1* and *Rht-D1*. This indicates that *Rht24* may become of great importance for the male pool in hybrid wheat breeding.

Although their diagnostic phenotype in the gibberellic acid seedling test has facilitated selection for the *Rht-1* semi-dwarfing genes in breeding programs, the use of GA-sensitive *Rht* genes has proven more difficult. In recent times molecular markers have provided an avenue to overcome this deficiency, but so far diagnostic markers are only available for the *Rht-1* loci that have been cloned. For *Rht8*, for example, Korzun *et al.* (1998) reported a certain SSR marker allele to be diagnostic for this gene, but this has more recently been shown to be strongly background dependent (Ellis *et al.*, 2007). Here, we demonstrate how markers in the *Rht24* chromosomal region can be converted into functional markers for a high-throughput analysis in breeding programs. It must be noted, however, that until *Rht24* is cloned, no truly diagnostic markers can be developed. Such markers targeting the *Rht24* region are nevertheless valuable for wheat breeding, as they allow *Rht24* to be tracked in progeny from a donor plant known to carry *Rht24b*. This can be achieved through single markers or the combination of several adjacent markers covering the *Rht24* region into haploblocks, which promise to be more broadly applicable and robust (Würschum *et al.*, 2017).

Future work is required to clone *Rht24*; however, as it belongs to the group of GA-sensitive genes, the evaluation of candidate genes should not be restricted to genes from the GA pathway. Cloning of the *Rht24* gene will reveal whether only one or several height-reducing *Rht24* alleles exist, i.e. will allow the establishment of a haplotype dictionary, as is already available for the *Rht-1* genes (Wilhelm *et al.*, 2013). Taken together, the characterization of *Rht24* presented in this work shows the relevance of this height-reducing gene in wheat worldwide, and thus expands the portfolio of *Rht* genes with commercial relevance in wheat breeding.

EXPERIMENTAL PROCEDURES

Plant materials and field experiments

A total of 1110 soft winter wheat (*T. aestivum* L.) cultivars were used for this study, as described previously (Würschum *et al.*, 2015b; Boeven *et al.*, 2016b). Worldwide genotypes are included, but the majority represent European cultivars released during the past decades. The experiment was conducted in a partially replicated design with a replication rate of 1.25 per location (Williams *et al.*, 2011), with 460 cultivars grown at three locations in the 2011/12 growing season, and with all 1110 cultivars grown at four locations in the 2012/13 growing season. Entries were sown in observation plots of two rows of 1.25 m in length. Plant height was recorded at full maturity and measured in cm from the ground to the tip of the spikes, excluding awns.

The phenotypic data were analyzed based on the following statistical model: $y_{ijkmo} = \mu + G_i + L_j + Y_k + LY_{jk} + GL_{ij} + GY_{ik} + GLY_{ijk} + r_{jkm} + b_{jkmo} + e_{ijkmo}$, where y_{ijko} was the phenotypic observation of the ith wheat line at the jth location in the kth year in the oth incomplete block of the mth replication, μ was an intercept term, G_i the genetic effect of the ith genotype, L_j the effect of the jth location, Y_k the effect of the kth year, GL_{ij} the genotype-by-location interaction, GY_{ik} the genotype-by-year interaction, GLY_{ijk} the genotype-by-location-by-year interaction, r_{jkm} the effect of the mth replication at the jth location in the kth year, b_{jkmo} the effect of the oth incomplete block of the mth replication at the jth location in the kth year, and e_{ijkmo} was the residual. Error variances were assumed to be heterogeneous among environments, i.e. location–year combinations. Variance components were determined by the restricted maximum likelihood (REML) method assuming a random model. The significance of variance component estimates was tested by model comparison with likelihood ratio tests. Best linear unbiased estimates (BLUEs) were estimated across environments assuming fixed effects for the genotype. Heritability (h^2) was estimated following the approach suggested by Piepho and Möhring (2007; formula 19). All statistical analyses were performed using ASREML-R 3.0 (Gilmour *et al.*, 2009).

Molecular markers and association mapping

Genotyping-by-sequencing (GBS) was employed for all lines at Diversity Arrays Technology (http://www.diversityarrays.com) using the Wheat GBS 1.0 assay. Markers with a minor allele frequency smaller than 0.05 were removed, resulting in a total of 23 720 markers for which a map position was available (Li *et al.*, 2015) and that were used for the analyses. CloneIDs of the silico DArT markers were prefixed with 'D' and those of the SNP markers were prefixed with 'S'. Genotyping of *Rht-B1* and *Rht-D1* followed the protocols reported by Ellis *et al.* (2002), and genotyping for *Ppd-D1* followed the protocols described by Beales *et al.* (2007).

For association mapping, an additive genetic model was chosen and mapping was performed with a mixed model incorporating a kinship matrix as described previously (Langer *et al.*, 2014). To control for multiple testing, we used a Bonferroni-corrected threshold of $P < 0.05$. The total proportion of genotypic variance (p_G) explained by the detected QTL was calculated by fitting all QTL and the segregating candidate genes simultaneously in a linear model, in the order of their strength of association. The ratio $p_G = R^2_{adj}/h^2$, where R^2_{adj} refers to the adjusted R^2 from the linear model, and 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 candidate genes and the QTL (SS_{QTL}) in this linear model. The allele substitution (α) effects were derived as the regression coefficient from models including just the marker under consideration.

The SSR markers *Xbarc103* and *Xwmc256* were assayed in a subset of 100 genotypes using their assays described on GrainGenes (https://wheat.pw.usda.gov).

Two SNP markers from the genome-wide assay (Table S2), located within the *Rht24* cluster of markers, were converted into KASP markers (http://www.lgcgroup.com/kasp), which are co-dominant, competitive allele-specific PCR-based markers. The KASP assay for marker S1066954 comprised the FAM-tailed primer (5′-FAM-tail-AGTGTCGGCATGACTGATTCTCC-3′) for the *Rht24a* wild-type allele, the HEX tailed primer (5′-HEX-tail-AGTGTCGGCATGACTGATTCTCA-3′) for the *Rht24b* height-reducing allele and the common primer (5′-CCAATCATCAAGGTGACTGTCATCA-3′). The KASP assay for marker S983322 comprised the FAM-tailed primer (5′-FAM-tail-CCCAGCGCAGTCTGTCCTG-3′) for the *Rht24b* height-reducing allele, the HEX tailed primer (5′-HEX-tail- AGCCCAGCGCAGTCTGTCCTA-3′) for the *Rht24a* wild-type allele and the common primer (5′-ACGCCATGGAAATTCTCGAGAT-3′). Both assays were run as 10-μl PCR reactions, with a standard KASP 65–57°C touchdown PCR program (http://www.lgcgroup.com/products/kasp-genotyping-chemistry/kasp-technical-resources) on a Roche LightCycler®480II instrument.

Gibberellic acid sensitivity assay

A set of 20 wheat genotypes (Table S3) carrying *Rht-B1b*, *Rht-D1b* and *Rht24b* alleles or wild-types for these *Rht* loci, were evaluated in a gibberellic acid sensitivity test. Fungicide-treated seeds were placed in paper towel rolls, with 10 seeds per roll. Each genotype was replicated four times per treatment, with one control (0 mg/L GA) and one GA treatment (50 mg/L GA, $C_{19}H_{22}O_6$). The two treatments were separated by using two different trays for the paper rolls. The prepared paper rolls were randomized per treatment in a randomized complete block design and kept at 4°C for 4 days. After 4 days the rolls were moved to a 20°C climate chamber with a long day (16-h light/8-h dark) regime. Each day 250 ml of either distilled water (control treatment) or 50 ppm GA (GA treatment) solution was given to each tray. After 10 days at 20°C the seedlings were measured from the seed until the first leaf node in mm.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Probable chromosomal positions of the candidate genes.

Figure S2. Characterization of the *Rht24* region on chromosome 6A.

Figure S3. Characterization of the *Rht24* region on chromosome 6A.

Figure S4. Development of KASP markers.

Table S1. Summary statistics for plant height.

Table S2. Results from association mapping.

Table S3. Phenotypic and QTL data of all cultivars.

REFERENCES

Archard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J.R. and Harberd, N.P. (2006) Integration of plant responses to environmentally activated phytohormonal signals. *Science*, **311**, 91–94.

Austin, R.B. (1980) Physiological limitations of cereal yields and ways of reducing them by breeding. In *Opportunities for Increasing Crop Yields*. (Hurd, R.G., Biscoe, P.V. and Dennis, C., eds). London: Pitman, pp. 3–19.

Austin, R.B., Ford, M.A. and Morgan, C.L. (1989) Genetic improvement in the yield of winter wheat: a further evaluation. *J. Agric. Sci.* **112**, 295–301.

Bai, C., Liang, Y. and Hawkesford, M.J. (2013) Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. *J. Exp. Bot.* **64**, 1745–1753.

Beales, J., Turner, A., Griffiths, S., Snape, J.W. and Laurie, D.A. (2007) A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* **115**, 721–733.

Boeven, P.H.G., Longin, C.F.H., Leiser, W.L., Kollers, S., Ebmeyer, E. and Würschum, T. (2016a) Genetic architecture of male floral traits required for hybrid wheat breeding. *Theor. Appl. Genet.* **129**, 2343–2357.

Boeven, P.H.G., Longin, C.F.H. and Würschum, T. (2016b) A unified framework for hybrid breeding and the establishment of heterotic groups in wheat. *Theor. Appl. Genet.* **129**, 1231–1245.

Börner, A., Worland, A.J., Plaschke, J., Schumann, E. and Law, C.N. (1993) Pleiotropic effects of genes for reduced height (*Rht*) and day-length insensitivity (*Ppd*) on yield and its components for wheat grown in middle Europe. *Plant Breed.* **111**, 204–216.

Botwright, T.L., Rebetzke, G.J., Condon, A.G. and Richards, R.A. (2001) The effect of *rht* genotype and temperature on coleoptile growth and dry matter partitioning in young wheat seedlings. *Aust. J. Plant Physiol.* **28**, 417–423.

Buerstmayr, M. and Buerstmayr, H. (2015) Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo × Arina. *Theor. Appl. Genet.* **128**, 1519–1530.

Buerstmayr, M. and Buerstmayr, H. (2016) The semidwarfing alleles *Rht-D1b* and *Rht-B1b* show marked differences in their associations with anther-retention in wheat heads and with fusarium head blight susceptibility. *Phytopathology*, **106**, 1544–1552.

Ellis, M.H., Bonnett, D.G. and Rebetzke, G.J. (2007) A 192bp allele at the Xgwm261 locus is not always associated with the Rht8 dwarfing gene in wheat (Triticum aesitvum L.). *Euphytica*, **157**, 209–214.

Ellis, M., Spielmeyer, W., Gale, K., Rebetzke, G. and Richards, R. (2002) "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theor. Appl. Genet.* **105**, 1038–1042.

Gilmour, A.R., Gogel, B., Cullis, B. and Thompson, R. (2009) *ASReml User Guide Release 3.0*. Hemel Hempstead, UK: VSN International Ltd.

Griffiths, S., Simmonds, J., Leverington, M., Wang, Y., Fish, L., Sayers, L., Alibert, L., Orford, S., Wingen, L. and Snape, J. (2012) Meta-QTL analysis

of the genetic control of crop height in elite European winter wheat germplasm. *Mol. Breeding*, **29**, 159–171.

Haque, M.A., Martinek, P., Watanabe, N. and Kuboyama, T. (2011) Genetic mapping of gibberellic acid-sensitive genes for semidwarfism in durum wheat. *Cereal Res. Commun.* **39**, 171–178.

Hay, R.K.M. (1995) Harvest index: a review of its use in plant breeding and crop physiology. *Ann. Appl. Biol.* **126**, 197–216.

He, X., Singh, P.K., Dreisigacker, S., Singh, S., Lillemo, M. and Duveiller, E. (2016) Dwarfing genes *Rht-B1b* and *Rht-D1b* are associated with both type I FHB susceptibility and low anther extrusion in two bread wheat populations. *PLoS ONE*, **11**, e0162499.

Hedden, P. (2003) The genes of the Green Revolution. *Trends Genet.* **19**, 5–9.

Korzun, V., Röder, M.S., Ganal, M.W., Worland, A.J. and Law, C.N. (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* **96**, 1104–1109.

Langer, S.M., Longin, C.F.H. and Würschum, T. (2014) Flowering time control in European winter wheat. *Front. Plant Sci.* **5**, 537.

Law, C.N., Snape, J.W. and Worland, A.J. (1973) The genetical relationship between height and yield in wheat. *Heredity*, **40**, 133–151.

Li, H., Vikram, P., Singh, R.P. *et al.* (2015) A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. *BMC Genom.* **16**, 216.

Lu, Q., Lillemo, M., Skinnes, H., He, X., Shi, J., Ji, F., Dong, Y. and Bjørnstad, Å. (2013) Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line 'Shanghai-3/Catbird'. *Theor. Appl. Genet.* **126**, 317–334.

McIntosh, R.A., Yamazaki, Y., Dubcovsky, J., Rogers, J., Morris, C., Appels, R. and Xia, X.C. (2013) Catalogue of gene symbols for wheat. Downloaded from http://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp on 01. Aug. 2016.

McIntosh, R.A., Dubcovsky, J., Rogers, W.J., Morris, C. and Xia, X.C. (2017) Catalogue of gene symbols for wheat: 2017 supplement. Downloaded from http://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp on 02. Feb. 2017.

Miedaner, T. and Voss, H.H. (2008) Effect of dwarfing *Rht* genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. *Crop Sci.* **48**, 2115–2122.

Piepho, H.P. and Möhring, J. (2007) Computing heritability and selection response from unbalanced plant breeding trials. *Genetics*, **177**, 1881–1888.

Rebetzke, G.J., Appels, R., Morrison, A.D., Richards, R.A., McDonald, G., Ellis, M.H., Spielmeyer, W. and Bonnett, D.G. (2001) Quantitative trait loci on chromosome 4B for coleoptile length and early vigour in wheat. *Aust. J. Agric. Res.* **52**, 1221–1234.

Rebetzke, G.J., Richards, R.A., Fettel, N.A., Long, M., Condon, A.G., Forrester, R.I. and Botwright, T.L. (2007) Genotypic increases in coleoptile length improves stand establishment, vigour and grain yield of deep-sown wheat. *Field Crops Res.* **100**, 10–12.

Reif, J.C., Maurer, H.P., Korzun, V., Ebmeyer, E., Miedaner, T. and Würschum, T. (2011) Mapping QTLs with main and epistatic effects underlying grain yield and heading time in soft winter wheat. *Theor. Appl. Genet.* **123**, 283–292.

Spielmeyer, W., Hyles, J., Joaquim, P., Azanza, F., Bonnett, D., Ellis, M.E., Moore, C. and Richards, R.A. (2007) A QTL on chromosome 6A in bread wheat (*Triticum aestivum*) is associated with longer coleoptiles, greater seedling vigour and final plant height. *Theor. Appl. Genet.* **115**, 59–66.

Srinivasachary, , Gosman, N., Steed, A., Simmonds, J., Leverington-Waite, M., Wang, Y., Snape, J. and Nicholson, P. (2008) Susceptibility to *Fusarium* head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. *Theor. Appl. Genet.* **116**, 1145–1153.

Tian, X., Wen, W., Xie, L. *et al.* (2017) Molecular mapping of reduced plant height gene *Rht24* in bread wheat. *Front. Plant Sci.* **8**, 1379.

Utz, H.F., Melchinger, A.E. and Schön, C.C. (2000) Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. *Genetics*, **154**, 1839–1849.

Vikhe, P., Patil, R., Chavan, A., Oak, M. and Tamhankar, S. (2017) Mapping gibberellin-sensitive dwarfing locus *Rht18* in durum wheat and development of SSR and SNP markers for selection in breeding. *Mol. Breed.* **37**, 28.

Wang, S., Wong, D., Forrest, K. *et al.* (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotech. J.* **12**, 787–796.

Whitford, R., Fleury, D., Reif, J.C., Garcia, M., Okada, T., Korzun, V. and Langridge, P. (2013) Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. *J. Exp. Bot.* **64**, 5411–5428.

Wilhelm, E.P., Mackay, I.J., Saville, R.J., Korolev, A.V., Balfourier, F., Greenland, A.J., Boulton, M.I. and Powell, W. (2013) Haplotype dictionary for the *Rht-1* loci in wheat. *Theor. Appl. Genet.* **126**, 1733–1747.

Williams, E., Piepho, H.-P. and Whitaker, D. (2011) Augmented p-rep designs. *Biom. J.* **53**, 19–27.

Worland, T. and Law, C.N. (1985) An effect of temperature on the fertility of wheats containing the dwarfing genes *Rht1*, *Rht2* and *Rht3*. Annual Rep Plant Breeding Inst, Cambridge 1984, 69–71.

Worland, T. and Snape, J.W. (2001) Genetic basis of worldwide wheat varietal improvement. In *The world wheat book – a history of wheat breeding*, vol **1**, (Bonjean, A.P. and Angus, W.J., eds). Paris: Lavoisier publishing, pp. 59–100.

Würschum, T., Langer, S.M. and Longin, C.F.H. (2015a) Genetic control of plant height in European winter wheat cultivars. *Theor. Appl. Genet.* **128**, 865–874.

Würschum, T., Boeven, P.H.G., Longin, C.F.H. and Leiser, W.L. (2015b) Multiply to conquer: copy number variations at *Ppd-B1* and *Vrn-A1* facilitate global adaptation in wheat. *BMC Genet.* **16**, 96.

Würschum, T., Leiser, W.L., Weissmann, S. and Maurer, H.P. (2017) Genetic architecture of male fertility restoration of *Triticum timopheevii* cytoplasm and fine-mapping of the major restorer locus *Rf3* on chromosome 1B. *Theor. Appl. Genet.* **130**, 1253–1266.

Yang, Z., Zheng, J., Liu, C., Wang, Y., Condon, A.G., Chen, Y. and Hu, Y.-G. (2015) Effects of the GA-responsive dwarfing gene *Rht18* from tetraploid wheat on agronomic traits of common wheat. *Field Crops Res.* **183**, 92–101.