



Molecular Mapping of Reduced Plant Height Gene *Rht24* in Bread Wheat

Xiuling Tian^{1,2}, Weie Wen^{1,2}, Li Xie², Luping Fu², Dengan Xu², Chao Fu², Desen Wang², Xinmin Chen², Xianchun Xia^{1,2}, Quanjia Chen¹, Zhonghu He^{2,3*} and Shuanghe Cao^{2*}

¹ College of Agronomy, Xinjiang Agricultural University, Urumqi, China, ² Institute of Crop Science, National Wheat Improvement Center, Chinese Academy of Agricultural Sciences, Beijing, China, ³ International Maize and Wheat Improvement Center (CIMMYT), Beijing, China

OPEN ACCESS

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*Correspondence:

Shuanghe Cao
shcao8@163.com
Zhonghu He
zhhecaas@163.com

Specialty section:

This article was submitted to
Crop Science and Horticulture,
a section of the journal
Frontiers in Plant Science

Received: 14 January 2017

Accepted: 24 July 2017

Published: 08 August 2017

Citation:

Tian X, Wen W, Xie L, Fu L, Xu D,
Fu C, Wang D, Chen X, Xia X,
Chen Q, He Z and Cao S (2017)
Molecular Mapping of Reduced Plant
Height Gene *Rht24* in Bread Wheat.
Front. Plant Sci. 8:1379.
doi: 10.3389/fpls.2017.01379

Height is an important trait related to plant architecture and yield potential in bread wheat (*Triticum aestivum* L.). We previously identified a major quantitative trait locus *QPH.caas-6A* flanked by simple sequence repeat markers *Xbarc103* and *Xwmc256* that reduced height by 8.0–10.4%. Here *QPH.caas-6A*, designated as *Rht24*, was confirmed using recombinant inbred lines (RILs) derived from a Jingdong 8/Aikang 58 cross. The target sequences of *Xbarc103* and *Xwmc256* were used as queries to BLAST against International Wheat Genome Sequence Consortium database and hit a super scaffold of approximately 208 Mb. Based on gene annotation of the scaffold, three gene-specific markers were developed to genotype the RILs, and *Rht24* was narrowed to a 1.85 cM interval between *TaAP2* and *TaFAR*. In addition, three single nucleotide polymorphism (SNP) markers linked to *Rht24* were identified from SNP chip-based screening in combination with bulked segregant analysis. The allelic efficacy of *Rht24* was validated in 242 elite wheat varieties using *TaAP2* and *TaFAR* markers. These showed a significant association between genotypes and plant height. *Rht24* reduced plant height by an average of 6.0–7.9 cm across environments and were significantly associated with an increased TGW of 2.0–3.4 g. The findings indicate that *Rht24* is a common dwarfing gene in wheat breeding, and *TaAP2* and *TaFAR* can be used for marker-assisted selection.

Keywords: CAPS marker, genome mining, *Rht24*, SNP chip, *Triticum aestivum*

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important food crops providing a significant proportion of the calories and protein consumed by humans (Mir et al., 2012; Chen et al., 2016). Grain yield in wheat largely depends on plant architecture, particularly plant height, which is significantly associated with biomass production and harvest index that ultimately determine yield potential (Law et al., 1978). Most importantly, appropriately reduced plant height reduces lodging and increases grain yield (Evans, 1998). The introduction of semi-dwarf varieties leading to the “Green Revolution” greatly enhanced crop yields globally (Peng et al., 1999).

To date, 23 dwarfing genes (*Rht1–Rht23*) have been cataloged in wheat (Supplementary Table S1) (McIntosh et al., 2015). Among them, only four homeologous genes *Rht1* (*Rht-B1b*), *Rht2* (*Rht-D1b*), *Rht3* (*Rht-B1c*), and *Rht10* (*Rht-D1c*) from the chromosome 4BS, 4DS, 4BS, and 4DS,

respectively, had been cloned (Peng et al., 1999; Pearce et al., 2011; Wu et al., 2011), 13 were on chromosomes 2AS (2), 2BL (1), 2DL (1), 3BS (1), 5AL (1), 5DL (1), 6AS (3), 7AS (1), and 7BS (2), but the locations of six other dwarfing genes have not been determined yet (Peng et al., 1999, 2011; Ellis et al., 2005; Wu et al., 2011; McIntosh et al., 2013; Chen et al., 2015). So far, more than 50 quantitative trait loci (QTL) for plant height have also been identified on all wheat chromosomes in previous reports (Börner et al., 2002; Peng et al., 2003; Quarrie et al., 2005; Liu et al., 2011; Griffiths et al., 2012; Würschum et al., 2015). For example, Wei et al. (2010) identified quite a few stable plant height QTL located on chromosomes 2A, 2B, 2D, 3B, 4B, 5A, 5D, 7B, and 7D. Griffiths et al. (2012) found that all chromosomes except for 3D, 4A, and 5D, had plant height genes using meta-QTL analysis. However, only a few genes for reduced stature have been used in wheat breeding because most showed negative effects on grain yield (Law et al., 1978; Worland et al., 1998; Chen et al., 2015). *Rht-B1b* (4BS), *Rht-D1b* (4DS) and *Rht8* (2DL) are extensively used in wheat breeding globally and molecular markers have been developed for their marker-assisted selection (MAS) (Korzun et al., 1998; Peng et al., 1999; Rebetzke and Richards, 2000; Ellis et al., 2002; Asplund et al., 2012). *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) were two major genes in the Green Revolution, and at present, approximately 70% of wheat varieties throughout the world contain at least one of them (Evans, 1998). *Rht8* reduced plant height around 10% and it has not significant negative effect on grain yield (Worland et al., 1998).

Simple sequence repeat (SSR) markers were favored for research and breeding in the last two decades due to high polymorphism, good repeatability, co-dominance and a simple polymerase chain reaction (PCR)-based system for analysis (Röder et al., 1998). With rapid advancements in sequencing technology, the quality of wheat genome assembly has been significantly improved and large numbers of high-quality scaffolds to facilitate gene isolation are available (Rogers, 2014). Simultaneously, many single nucleotide polymorphisms (SNPs) were identified for developing SNP chips, providing high-throughput genotyping platforms that have been extensively used for genome-wide association studies (GWAS) and QTL mapping at high resolution (Colasuonno et al., 2014; Wang et al., 2014; Sukumaran et al., 2015; Gao et al., 2016; Wu et al., 2016). Many QTL for agronomic traits, grain and industrial quality and disease resistance have been identified in wheat (Buerstmayr et al., 2009; Wang et al., 2014; Naruoka et al., 2015; Jin et al., 2016; Liu et al., 2016; Zhai et al., 2016). Thus, the availability of high quality wheat genome data and high-throughput SNP genotyping platforms greatly advanced wheat genetics and breeding.

We previously identified a plant height major QTL *QPH.caas-6A* between *Xwmc256* and *Xbarc103*, which explained 8.0–10.4% of the phenotypic variance across eight environments using an $F_{2:4}$ population derived from the Jingdong 8/Aikang 58 cross (Li et al., 2015). In addition, *QPH.caas-6A* probably was consistent with *QTL_height_6A_1*, which also linked with *Xwmc256* on chromosome 6AL and explained 6.3–29.1% of the phenotypic variance (PVE) (Griffiths et al., 2012). Furthermore, *QPH.caas-6A* was identified to reduce TGW 6.5–8.2%, increase

kernel number per spike and number of spike by 2.4–3.5% and NS by 2.0–4.6%, respectively, which is similar to “green revolution” gene *Rht2* (Li et al., 2015). Thus *QPH.caas-6A* was a potentially useful dwarfing locus in wheat breeding. Here we designate *QPH.caas-6A* as *Rht24*. The previously closest markers flanking *Rht24* were *Xbarc103* and *Xwmc256*, which were 8.2 cM apart. The aim of the present study was to identify markers more closely linked to *Rht24* using the wheat genomic database and a 660K SNP chip and thereby establish a more efficient MAS system for wheat breeding.

MATERIALS AND METHODS

Plant Materials

Two hundred and fifty-six recombinant inbred lines (RILs, $F_{2:6}$) from a cross between Aikang 58 (AK58) × Jingdong 8 (JD8) were used for genetic analysis. AK58, a leading variety occupying more than one million ha in the Yellow and Huai Valley, has a short plant height and excellent lodging resistance. Jingdong 8, with relatively tall plant height and excellent resistance to heat during the grain filling stage, was an elite variety in the Northern China Plain Region. Two sets of Chinese and introduced elite varieties (Supplementary Tables S4, S5), comprising 154 (Set I) and 88 (Set II) varieties, were used to validate the efficacy of MAS for *Rht24* and to investigate allelic distributions.

Field Trials and Phenotype Evaluation

The RILs and parents were planted at Gaoyi in Hebei province and Anyang in Henan during the 2014–2015 cropping season, and at Gaoyi during 2015–2016 cropping season. The experimental design was randomized complete blocks and three replications. Each plot was a single 2 m row with 25 cm between rows.

Set I varieties were planted at Anyang in Henan province and Suixi in Anhui, respectively, during the 2012–2013 and 2013–2014 cropping seasons. Set II varieties were grown at Shijiazhuang in Hebei and Beijing, respectively, during the 2012–2013 and 2013–2014 cropping seasons. These were grown in randomized complete blocks with three replications. Each plot consisted of four 2 m rows spaced 30 cm apart and approximately 50 plants in each row. Field management was according to local practice. During the whole wheat growth period in our field trial, there is no extreme weather causing serious damages, such as cold spell in later spring, dry and hot wind in filling stage, and fertilization (bottom fertilizer including 150 kg/h² Urea plus 400 kg/h² Diammonium Phosphate; 300 kg/h² Urea just before elongation stage) is enough for wheat growth and development.

Plant height was measured from the ground to spike (awns excluded) at grain-filling. For each plot, five representative primary tillers on different plants in the middle of each row were selected to measure the plant height, and the averaged value was used for subsequent analysis. TGW was determined by weighing triplicate 200 grain samples.

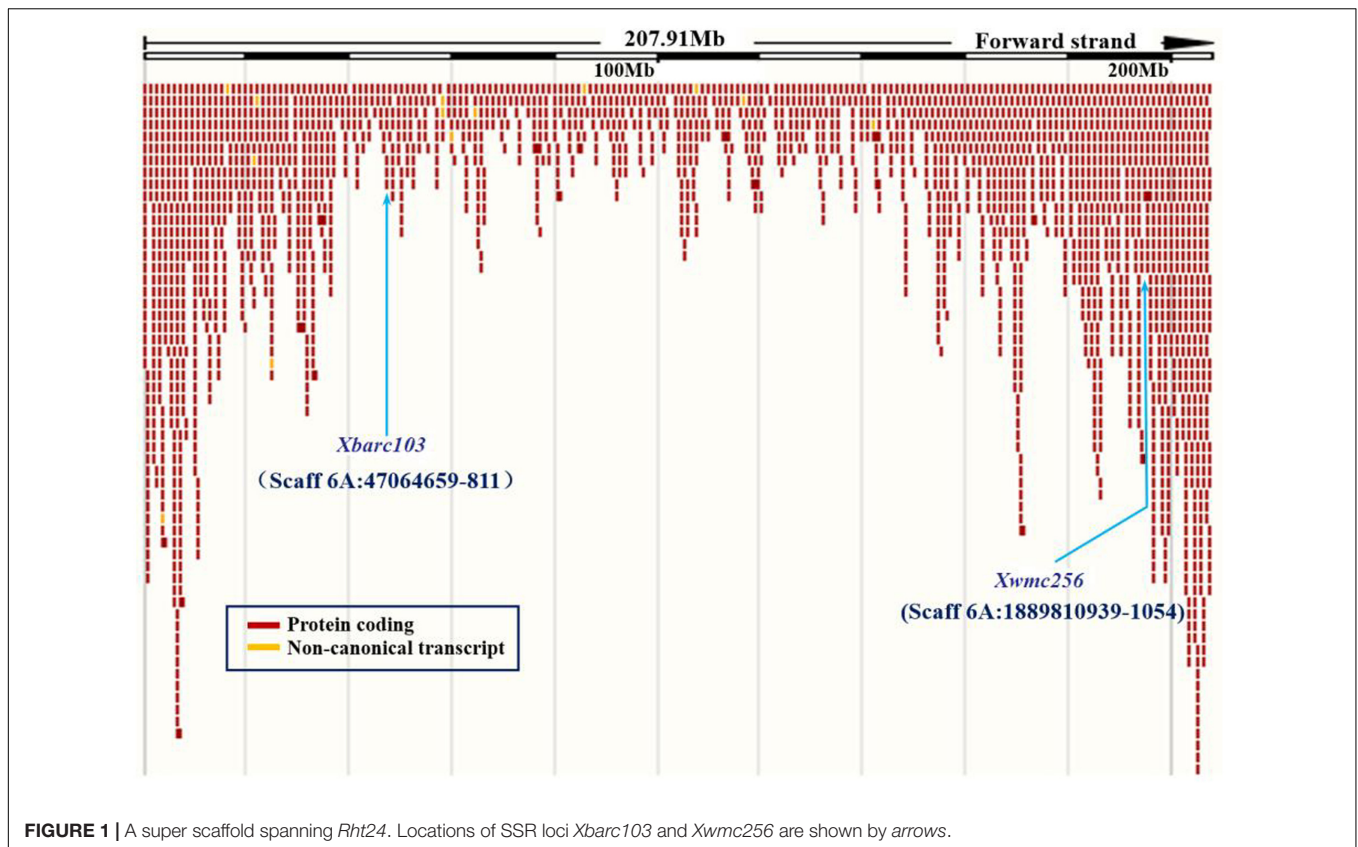


FIGURE 1 | A super scaffold spanning *Rht24*. Locations of SSR loci *Xbarc103* and *Xwmc256* are shown by arrows.

Genotypic Analysis Using Simple Sequence Repeat (SSR) Markers

Genomic DNA was isolated from young leaves using the modified CTAB method (Saghai-Maroo et al., 1984). Twenty-two SSR markers on chromosome 6AL were chosen for genetic analyses¹ (Supplementary Table S7).

Polymerase chain reaction was performed in a 15 μ l reaction system containing 7.5 μ l of 2 \times Taq PCR Mix (Tianwei Biotechnology Co., Ltd., Beijing²), 5 pmol of each primer and 100 ng of genomic DNA. Amplification was performed at 94°C for 5 min, followed by 35 cycles at 94°C for 20 s, 50–60°C (depending on specific primers) for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR products were separated in 6% polyacrylamide or 2% agarose gels.

Gene-Specific Marker Development by Genome Mining Approach

To confirm the target sequences of the SSR mentioned above, PCR products were purified using the TIANgel MIDI Purification Kit (Tiangen, Biotechnology Co., Ltd., Beijing³). The purified PCR products were ligated with cloning vector pEASY-T5 Zero and then transformed into Trans1-T1 competent cells by the

heat shock method (TransGen Biotech Co., Ltd., Beijing⁴). At least three positive clones from each transformation were randomly selected and sequenced at Shanghai Sangon Biotech Co., Ltd⁵. The SSR target sequences were used as BLAST queries⁶ to identify desirable scaffolds. The genes of interest in the target scaffold were isolated and sequenced following the above procedure. Sequence sites polymorphic between the parents were identified by alignments with DNAMAN software⁷ for designing gene-specific markers. Cleaved amplified polymorphic sequences (CAPS) or derived CAPS (dCAPS) marker for each target gene was developed following Thiel et al. (2004).

SNP Chip-Based Screening

Wheat 660K SNP chip was developed by the Institute of Crop Science, Chinese Academy of Agricultural Sciences, synthesized by Affymetrix and commercially available at CapitalBio Corporation⁸. Recently, the chip has been efficiently used in our lab (Jin et al., 2016).

To identify more molecular markers linked to *Rht24* the 660K SNP chip was used to test two parents and two contrasting bulks of the RIL population, comprising equal amounts of

¹<http://wheat.pw.usda.gov>

²<http://www.tw-biotech.com>

³<http://tiangen.casart.com.cn>

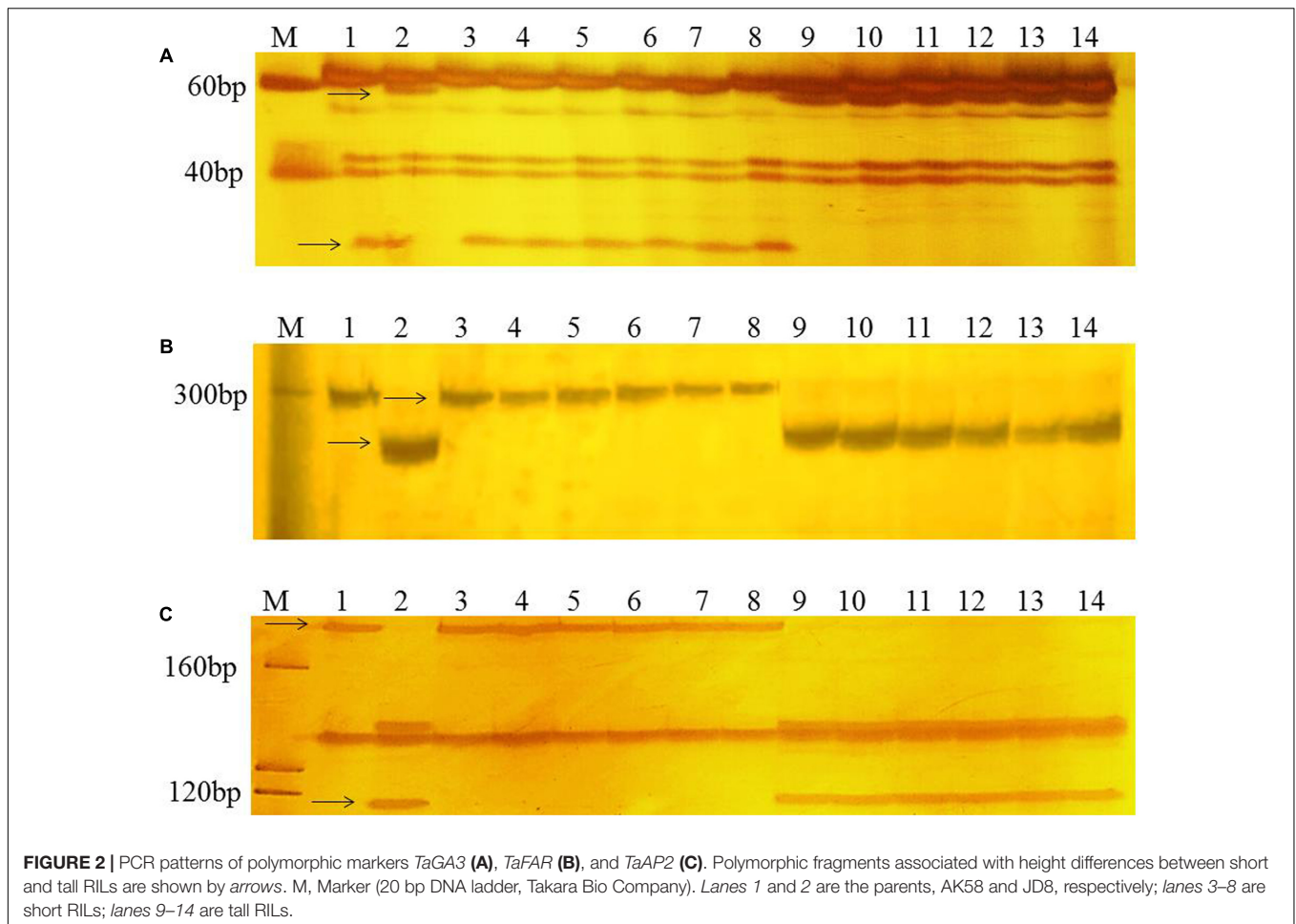
⁴<http://www.transgen.com.cn>

⁵<http://www.sangon.com/>

⁶http://plants.ensembl.org/Triticum_aestivum/Info/Index

⁷<http://www.lynnon.com>

⁸http://wheat.pw.usda.gov/ggpapes/topics/Wheat660_SNP_array_developed_by_CAAS.pdf



genomic DNA from 10 tall and 10 short RILs, respectively. Genotyping was performed at CapitalBio Technology Company⁹. SNPs polymorphic between parents and between two bulks were selected to develop CAPS markers.

Genetic Linkage Map Construction and Statistical Analysis

Quantitative trait locus IciMapping 4.0¹⁰ was used for linkage map construction and QTL analysis using a LOD score threshold of 3.0 (Lincoln et al., 1992). Plant height was reformed with an algorithm SERiation and criterion SARF (sum of adjacent recombination frequencies). Variance analysis and *t*-test (Duncan method) were conducted with SAS 9.4¹¹. The broad-sense heritability (h_B^2) of the corresponding traits was calculated using the following formula:

$$h_B^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ge}^2/e + \sigma_{\epsilon}^2/re),$$

where σ_g^2 , σ_{ge}^2 , and σ_{ϵ}^2 were estimates of lines, line \times environment interactions and residual error variances,

⁹<http://www.capitalbiotech.com>

¹⁰<http://www.isbreeding.net/>

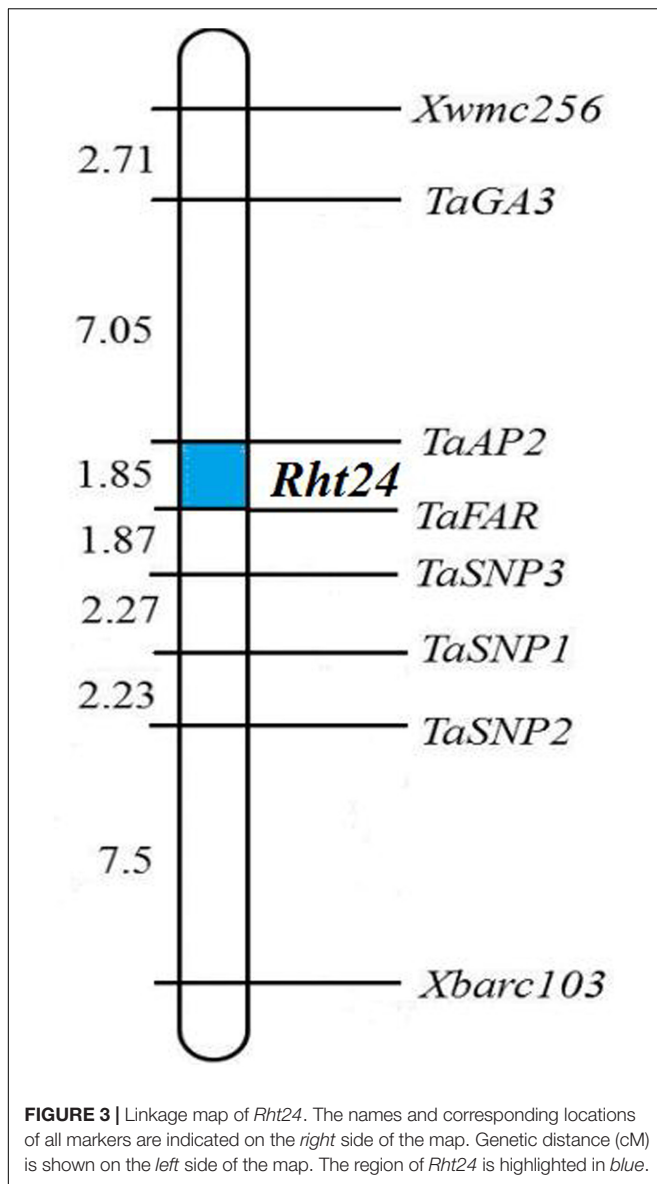
¹¹<http://www.sas.com>

respectively, and e and r represented the numbers of environments and replicates, respectively (Supplementary Table S6; Nyquist and Baker, 1991). *T*-test was used to perform the association analysis between *Rht24* and plant height as well as TGW.

RESULTS

Confirmation of *Rht24*

In our previous study, *Rht24* was identified with LOD score of approximately 12, explaining 8.0–10.4% of the phenotypic variance; it was mapped within an 8.2 cM interval flanked by *Xbarc103* and *Xwmc256* (Li et al., 2015). To further verify *Rht24*, 22 SSR markers were selected from the targeted region of chromosome 6AL (Supplementary Table S7). Among them, only *Xbarc103* and *Xwmc256* were polymorphic between the parental lines and bulk lines built by 10 tall and 10 short lines (Supplementary Figure S1). *Rht24* was located between the two markers, which were 19.46 cM apart. Although the genetic distance was greater than reported previously, the QTL for reduced plant height was confirmed.



Narrow-Down of *Rht24* by the Genome Mining Approach

To confirm the chromosomal location of *Rht24*, we cloned and sequenced the differential fragments of *Xbarc103* and *Xwmc256* in the tall and short lines, respectively (Supplementary Figure S1). Sequencing results showed that they were consistent with the reported sequences¹². Using the sequences as queries to BLAST against International Wheat Genome Sequence Consortium database¹³, a super scaffold of approximately 208 Mb (approximately 1200 genes) spanning *Rht24* was identified (Figure 1). Twenty genes involved in plant morphogenesis and hormone metabolism were selected from the scaffold and sequenced (Supplementary Table S8). Polymorphisms

¹²<http://wheat.pw.usda.gov>

¹³http://plants.ensembl.org/Triticum_aestivum/Info/Index

between AK58 and JD8 were detected at loci *TaGA3*, *TaFAR*, and *TaAP2* (Supplementary Figures S2A–C and Table S2). Based on these polymorphisms, three gene-specific CAPS or dCAPS markers were developed (Figures 2A–C and Supplementary Figures S2A–C). Upon genotyping the RIL, the location of *Rht24* was narrowed to a 1.85 cM interval flanked by *TaFAR* and *TaAP2* (Figure 3).

Enriching Molecular Markers in the *Rht24* Region by a Combination of SNP Genotyping Assays and Genomic Identification

To enrich molecular markers in the *Rht24* region, the 660K SNP chip was used in genotyping assays. In total, more than 400 polymorphic SNPs on chromosome 6A from wheat 660K SNP chips were identified between two contrasting bulks (data not shown). Among them, the target sequences of polymorphic SNPs anchored in the super scaffold above were used to develop site-specific CAPS markers (Supplementary Table S9). Among them, three polymorphic loci, *TaSNP1*, *TaSNP2*, and *TaSNP3*, were mapped in the region of *Rht24* (Supplementary Figures S3A–C, S4A–C, Table S2 and Figure 3). However, they did not further narrow down the *Rht24* locus.

Validation of Molecular Markers Closely Linked to *Rht24*

Among 242 wheat varieties genotyped by markers *TaFAR* and *TaAP2* (Supplementary Figures S5A,B), *Rht24* was present in 185 (76.4%) varieties and absent in 41 (17%) (Supplementary Table S3). The average plant height for the varieties containing *Rht24* was 78.2 and 80.2 cm in Sets I and II, respectively, whereas for those without *Rht24* the comparative values were 86.1 and 86.2 cm, respectively, indicating that varieties with *Rht24* were generally shorter than those without ($P_I = 0.029$, $P_{II} = 0.003$, Table 1). *Rht24* was also significantly associated with thousand grain weight (TGW) in Set I ($P = 0.014$, Table 2). Although *Rht24* had no significant association with TGW in Set II, the average TGW of entries with *Rht24* was 2 g higher than those without ($P = 0.196$, Table 2). On average *Rht24* reduced plant height by 6.0–7.9 cm and increased TGW by 2.0–3.4 g in all varieties used in this study (Tables 1, 2).

DISCUSSION

Rht24 Is an Important Locus Affecting Plant Height and Grain Weight in Wheat

Previous studies showed that loci controlling plant height were present in all 21 wheat chromosomes (Mccartney et al., 2005; Gale and Law, 1973; Griffiths et al., 2012). The QTL *QTL_height_6A_1* linked with *Xwmc256* on chromosome 6AL explained 6.3–29.1% of the phenotypic variance (PVE) (Griffiths et al., 2012). *Rht24*, previously designated as *QPH.caas-6A*, was flanked by SSR markers *Xwmc256* and *Xbarc103* (Li et al., 2015), indicating that it could be the same as *QTL_height_6A_1*. Here we developed two markers, *TaFAR* and *TaAP2* that closely flanked *Rht24*. The

TABLE 1 | Association analysis between *Rht24* genotypes and plant height in two sets of germplasm.

Germplasm	Year	Environment	Genotype ^a	Number of accessions	Mean plant height (cm) ^b	SD (cm)	Range (cm)	
Set I	2012–2013	Anyang	A	17	88.5a	14.7	75.0–138.3	
			B	128	81.0a	7.0	63.3–106.7	
		Suixi	A	17	84.8a	12.4	71.0–125.0	
			B	128	77.2b	6.6	55.0–95.7	
	2013–2014	Anyang	A	17	83.6a	15.2	66.0–135.7	
			B	128	74.4b	7.0	58.2–92.1	
		Suixi	A	17	87.8a	12.4	72.4–125.8	
			B	128	80.2b	6.6	59.8–100.9	
	Average	A	17	86.1a	13.5	72.2–131.2		
		B	128	78.2b	6.4	59.5–98.8		
	Set II	2012–2013	Beijing	A	24	82.3a	10.3	62.9–109.8
				B	57	76.0b	8.0	49.3–93.9
Shijiazhuang			A	24	85.3a	9.0	68.2–108.4	
			B	57	80.5b	7.6	55.6–97.3	
2013–2014		Beijing	A	24	82.3a	10.3	62.9–109.8	
			B	57	76.0b	8.0	49.3–93.9	
		Shijiazhuang	A	24	85.3a	9.0	68.2–108.4	
			B	57	80.5b	7.6	55.6–97.3	
Average		A	24	86.2a	9.5	67.8–110.7		
		B	57	80.2b	7.5	57.7–95.7		

^aA, *JD8* parental genotypes and B, *AK58* parental genotypes based on flanking markers *TaAP2* and *TaFAR*. Varieties with recombinant genotypes are not included.

^bDifferent lowercase letters following the mean plant height indicate significant differences between two genotypes at $P < 0.05$ (Dunnett's *t*-test); SD, standard deviation.

TABLE 2 | Association analysis between *Rht24* genotypes and TGW in two sets of germplasm.

Germplasm	Year	Environment	Genotype ^a	Number of accessions	Mean TGW (g) ^b	SD (g)	Range (g)	
Set I	2012–2013	Anyang	A	17	38.7a	6.0	30.1–53.3	
			B	128	41.4b	5.0	25.3–56.1	
		Suixi	A	17	39.2a	6.1	29.3–49.8	
			B	128	42.7b	5.5	25.7–56.3	
	2013–2014	Anyang	A	17	47.0a	6.9	35.3–59.7	
			B	128	50.3b	5.2	28.3–63.4	
		Suixi	A	17	42.4a	7.4	30.2–57.7	
			B	128	46.3b	5.7	22.5–62.2	
	Average	A	17	41.8a	6.3	31.7–54.0		
		B	128	45.2b	5.0	26.1–59.2		
	Set II	2012–2013	Beijing	A	24	30.7a	6.6	21.5–43.3
				B	57	32.2a	5.5	21.4–42.9
Shijiazhuang			A	24	32.3a	7.1	20.9–48.1	
			B	57	34.1a	6.0	21.1–46.3	
2013–2014		Beijing	A	24	29.6a	6.0	20.5–44.4	
			B	57	30.9a	4.9	19.7–40.1	
		Shijiazhuang	A	24	39.1a	9.4	25.5–66.1	
			B	57	42.3a	7.7	26.3–56.3	
Average		A	24	32.9a	7.1	22.1–50.5		
		B	57	34.9a	5.7	22.5–46.1		

^aA, *FAR-a/AP2-a* (parental *JD8* genotype); B, *FAR-b/AP2-b* (parental *AK58* genotype). ^bDifferent lowercase letters following the mean plant height indicate significant differences between two genotypes at $P < 0.05$ (Dunnett's *t*-test); SD, standard deviation.

two markers were used to test the genetic effect of *Rht24* and observed similar result with the previously reported (Table 3; Griffiths et al., 2012; Li et al., 2015). *Rht24* genotyped by *TaAP2*

and *TaFAR* as markers showed a significant association with reduced height among elite varieties. It was present in about 76% of elite varieties indicating that it was positively selected in wheat

TABLE 3 | Phenotypic effect of *Rht24* on plant height in the AK58/JD8 RIL population.

Environment	Interval	LOD ^a	Add ^b	PVE (%) ^c
Anyang (2014–2015)	TaAP2-TaFAR	4.6	-3.1	9.9
Gaoyi (2014–2015)	TaAP2-TaFAR	11.7	-3.9	23.7
Gaoyi (2015–2016)	TaAP2-TaFAR	5.6	-3.1	10.7
Average	TaAP2-TaFAR	8.0	-3.4	16.8

^aLogarithm of odds score; QTLs were detected at a LOD threshold of 3.0. ^bAdditive effects. ^cPercentage of phenotypic variance explained by *Rht24*.

breeding programs (Supplementary Table S3). In our previous study, *Rht24* reduced TGW by 6.9–8.5%. However, *Rht24* had a positive effect on thousand kernel weight in the present study (Table 2). We speculate that two reasons probably account for this case. The AK58/JD8 RIL and natural populations have better hereditary stability than the previous $F_{2:4}$ mapping population and are used to define *Rht24* with more accurate effect analyses. Additionally, the interval of *Rht24* was narrowed down and thus more interference effects of other genes were excluded using the closest flanking markers, *TaFAR* and *TaAP2*. In fact, it still cannot be ruled out that other genes disturb the effect of *Rht24* on TGW based on our existing mapping information. Thus it is necessary to further narrow down the interval of *Rht24*. Thus, *Rht24* is an important QTL for plant height and TGW in wheat and MAS mediated by selection for appropriate *TaAP2* and *TaFAR* alleles should be effective.

Distribution of *Rht-B1b*, *Rht-D1b*, and *Rht8* in Chinese germplasm was detected by molecular markers, with frequencies of 24.5, 45.5, and 46.8%, respectively (Zhang et al., 2006). In addition, *Rht-B1b* and *Rht-D1b* were tested by KASP assays in current Chinese leading varieties with frequencies of 36.9 and 38.6%, respectively (Rasheed et al., 2016). Our study showed that *Rht24* was present in about 76% of elite Chinese varieties, a much higher frequency than either *Rht-B1b* or *Rht-D1b* (Supplementary Table S3). The results indicated that *Rht24* had the highest frequency distribution compared with the other three loci, and it was frequently present in combination with *Rht-D1b* or *Rht8*. It was reported that *Rht8* not only to reduce plant height but also significantly increase TGW (Zhang et al., 2016), however, *Rht-B1b* and *Rht-D1b* showed negative effects on TGW (Li et al., 1998). In this study, *Rht24* increased TGW by 2.0–3.4 g in all varieties used (Table 2).

In all, *Rht24* was important and extensively used in Chinese wheat breeding programs. Further study is needed to understand the origin of *Rht24* in Chinese wheat and investigate *Rht24* effect on the other traits, such as tiller number, flowering date, spike length and so on.

Combination of SNP Chip-Based Screening and Genome Mining Was an Effective Approach for Fine Mapping of *Rht24*

As the development of genome sequencing technology, high-quality wheat genome sequencing data has been continuously

updated and released¹⁴. Based on BLAST against wheat genome databases, the markers from preliminary mapping can be used as entry points to mine new markers linked to the loci of interest. In the present study we used SSR markers flanking *Rht24* as queries and identified a super scaffold. According to gene annotations, we developed three gene-specific markers and narrowed the region of *Rht24* from a 19.46 cM interval (8.2 cM in our previous study) to 1.8 cM. A 660K SNP chip was recently developed and released¹⁵. The 660K chip has several advantages compared to the 90K SNP chip, such as higher marker density and higher resolution as well as better distribution on chromosomes. We used this chip to screen candidate markers linked to *Rht24* and a significant number of polymorphic SNPs were obtained (data not shown). It was an obviously effective approach to take advantage of constantly updated genome information and high-throughput SNP platforms.

ETHICS STATEMENT

We declare that these experiments comply with the ethical standards in China.

AUTHOR CONTRIBUTIONS

XT, LX, and DX performed the experiments. XT, WW, LF, CF, DW, XC, and QC did the field trials. SC and ZH designed the experiment. XT, SC, XX, and ZH wrote the paper.

FUNDING

This study was supported by the National Key Research and Development Program of China (2016YFD0101802, 2016YFE0108600) and the National Natural Science Foundation of China (31161140346, 31461143021, 31371623).

ACKNOWLEDGMENT

The authors are grateful to Prof. R. A. McIntosh, Plant Breeding Institute, University of Sydney, for critical review of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fpls.2017.01379/full#supplementary-material>

¹⁴http://plants.ensembl.org/Triticum_aestivum

¹⁵http://wheat.pw.usda.gov/ggpages/topics/Wheat660_SNP_array_developed_by_CAAS.pdf

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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