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A 36 Mb terminal deletion of chromosome 2BL is responsible for a wheat semi-dwarf mutation

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ABSTRACT

Reduced plant height is one of the most important traits related to lodging resistance and crop yield. The use of reduced height genes has been one of the main features in breeding modern high-yielding wheat varieties with less lodging. A spontaneous dwarf mutant DD399 was identified in a high yielding, gibberellic acid (GA)-insensitive, lodging-resistant variety Nongda 399 (ND399). Significant differences in upper internode lengths between mutant DD399 and wild type ND399 were caused by reduced cell elongation. The plant height of ND399 × DD399 F_1 hybrids was intermediate between the parents, indicating incomplete dominance or a dose–response effect of a reduced height gene. Plant height showed continuous distribution in the F_2 population, and segregation distortion was observed among the 2292 $F_{2:3}$ progenies. The reduced height mutation was characterized by Illumina 90 K iSelect SNP genotyping and bulked segregant RNA-Seq (BSR-Seq) analysis of the segregating population. A concentrated cluster of polymorphic SNPs associated with the reduced height phenotype was detected in the distal region of chromosome arm 2BL. Co-segregation of reduced height phenotype with the clustered markers revealed a 36 Mb terminal deletion of chromosome 2BL in mutant DD399.

1. Introduction

Common wheat, the most widely grown cereal grain crop, occupies about one-fifth of the total cultivated land in the world and provides more calories and protein in the human diet than any other crop. There is a strong relationship between biomass and plant height in most plant species [1] and a positive correlation between grain yield and plant height [2–6]. But the stems of tall plants tend to be too weak to support the grain in high-yielding varieties and lodging and grain loss is a frequent consequence. The Green Revolution in the 1960s was based on the use of reduced height genes in wheat and rice that enabled plants to respond to higher nutrient conditions without excessive lodging [7]. The new so-called semi-dwarf varieties not only had stiff straw and were not as prone to stem collapse before harvest but also had a greater proportion of assimilate partitioned into the grain, resulting in further yield increases [8,9]. Early in the 20th century, a reduced height wheat variety called 'Daruma' was crossed with American high-yielding varieties and produced 'Norin10′ which was used in breeding programs in the United States of America and International Wheat and Maize Improvement Center (CIMMYT) to develop high-yielding reduced height cultivars. The *Rht-B1b* and *Rht-D1b* alleles from 'Norin10′ were widely used thereafter in worldwide wheat breeding programs [8,10].

Plant height in bread wheat is generally considered to be a complex trait controlled by major reduced height and minor genes [11,12]. To date, 25 reduced height genes (*Rht1-Rht25*) have been cataloged in wheat [13–16]. Among them, *Rht1* (*Rht-B1b*), *Rht2* (*Rht-D1b*), *Rht3* (*Rht-B1c*), and *Rht10* (*Rht-D1c*) have been cloned [17–19] and Ellis et al. [20] developed specific PCR markers to detect the point mutations responsible for *Rht-B1b* and *Rht-D1b*. *Rht23* likely encodes a Q homeolog with pleiotropic effects on plant height and spike compactness [21]. *Rht-B1b*, *Rht-B1c*, *Rht-D1b*, and *Rht-D1c* are gibberellic acid 3 (GA3)-insensitive, whereas other genes such as *Rht4* to *Rht25* are GA3-sensitive [13–16]. In addition

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to reducing plant stature, *Rht-D1b* and *Rht-B1b* also reduce coleoptile length and seedling vigor. However, shorter coleoptile length and decreased seedling vigor were not found in varieties with GA_3-sensitive reduced height genes, such as *Rht8* and *Rht9* [22–24].

Although a number of reduced height genes have been characterized only a few of them have been widely applied in wheat improvement [25]. Based on GA reaction and pedigree analyses, Jia et al. [26] and Guo et al. [27] showed that the most extensively used reduced height genes in China were *Rht-B1b*, *Rht-D1b*, *Rht8*, and *Rht9*. *Rht24* was also frequently found in wheat cultivars from China and elsewhere [15]. To further enhance wheat yield potential and lodging resistance the search for new reduced height genes continues.

Gene mapping in common wheat is time-consuming and laborious due to the enormous genome size (~17 Gb), allohexaploidy (2*n* = 42, genomes AABBDD), and abundance of repetitive DNA. Next generation sequencing technology (NGS) provides abundant sequence data for development of single nucleotide polymorphism (SNP) markers linked to traits of interest via SNP arrays [28] and BSR-Seq [29]. For example, by high-throughput 90 K SNP array genotyping of pooled extremes Wu et al. [30] identified major effect adult plant stripe rust resistance QTL *Qyr.nwafu-6BL* in hexaploid wheat. Using Illumina 90 K iSelect SNP chip and bulked segregate analysis (BSA) Wu et al. [31] performed a large-scale detection of powdery mildew resistance genes in 36 wheat lines. Trick et al. [32] mapped the high grain protein content gene *GPC-B1* to a 0.4 cM interval in polyploid wheat by BSR-Seq analysis. By combining BSR-Seq and comparative genomics analyses the stripe rust resistance gene *YrZH22* was mapped to a 5.92 cM genetic interval spanning a 4.0 Mb physical genomic region on wheat chromosome 4BL [33].

In the present study, we report the identification of a GA_3-insensitive spontaneous reduced height mutant DD399 from the high yielding, lodging-resistant wheat cultivar Nongda 399. The reduced height mutation was mapped to a terminally deleted fragment of chromosome 2BL using Illumina 90 K SNP assay and BSR-Seq analysis.

2. Materials and methods

2.1. Materials

The DD399 mutant was identified during ear-to-row seed purification cultivar Nongda 399 (ND399, pedigree Torino/2*2552//9516/3/5*Shi 4185). A ND399 × DD399 cross was made to produce F_1, F_2, and derivative $F_{2:3}$ materials for mapping the reduced height gene. Chromosomal arm assignment and bin mapping of markers linked to the reduced height gene were conducted using Chinese Spring (CS) and CS deletion line 2BL6-0.80-1.00 (kindly provided by Drs. W.J. Raupp and B.S. Gill, Wheat Genetics Resource Center, Kansas State University, USA).

2.2. Field experiment and phenotype measurement

ND399 and DD399 were grown at Beijing, China (BJ, N40.08°, E116.10°) and Gaoyi Stock Seed Farm at Gaoyi in Hebei province (GY, N37.38°, E114.36°) during the 2010–2011 and 2013–2014 growing seasons for evaluation of phenotypic traits. The F_1 was evaluated at BJ in 2011–2012 and an F_2 population and $F_{2:3}$ families were evaluated at GY in 2012–2013 and 2013–2014, respectively. CS and CS deletion line 2BL6-0.89-1.00 were tested in 2014 at BJ. Plots were represented by 3 m rows sown with 40 seeds and a row spacing of 25 cm. Field management was the same as commonly practiced in wheat production.

Plant heights (from the surface of the soil to the tip of the main spike) of ND399, DD399, and F_1, F_2 individuals, $F_{2:3}$ rows, CS and del-2BL6-0.89-1.00 were recorded. Five plants were sampled for ND399, DD399, and the F_1 and three plants were recorded for CS and del-2BL6-0.89-1.00. Plant height of the main spike was recorded for each F_2 individual. Uniformity in plant height of $F_{2:3}$ families was used to verify the homozygosity versus heterozygosity of each F_2 individual. One representative plant was measured for each homozygous family, whereas the shortest and tallest plants were measured in segregating families. Thousand grain weight (TGW), main spike length (SL), and individual internode lengths of ND399, DD399, the F_1, CS, and del-2BL6-0.89-1.00 were also determined. TGW was recorded using an electronic balance to determine the average weight of three independent 100-grain samples. Effective tiller number (TN) and grain number of the main spike (GN) of ND399, DD399, and the F_1 were also measured. CS (tall) and Aibian 1 (*Rht-D1c*) were used as GA_3-sensitive and GA_3-insensitive controls.

2.3. Gibberellic acid 3 (GA_3) response test

ND399, DD399, CS (sensitive control), and Aibian 1 (insensitive control) were tested for response to foliar application of GA_3 at the seedling stage using the method reported by Wu et al. [19].

2.4. Microscopic analysis and cell dimensions

The central elongating section of main tiller peduncles of DD399 and ND399 were sampled at flowering for determination of cell dimensions. The peduncles were fixed in formalin-acetic alcohol (FAA) and vacuum-infiltrated overnight. These cut tissues were embedded in paraffin and transverse and longitudinal sections were stained with safranin and fast green, and then observed using an Olympus stereo fluorescence microscope (SEX16, Olympus, Japan).

2.5. Statistical analysis

Comparisons of tested traits was performed with GraphPad Prism 8.0.1 (GraphPad Software, San Diego, CA, USA). Chi-squared (χ^2) tests for goodness-of-fit were used to compare observed and theoretically expected segregation ratio.

2.6. Illumina iSelect 90 K SNP array for detection of genetic differences between DD399 and ND399

High-density SNP genotyping arrays containing 81,587 SNPs [28] were used to detect genetic differences between DD399 and ND399. SNP genotyping was conducted at the Genome Center of the University of California at Davis, USA, according to the manufacturer's protocols (Illumina, San Diego, CA, USA). SNP allele clustering and genotype calling was performed with GenomeStudio v2011.1 software as described in Cavanagh et al. [34].

2.7. BSR-Seq

BSR-Seq analysis as described in Wang et al. [33] was used to identify candidate SNP variations. Leaves of 60 extremely tall and 60 extremely short F_2 plants were pooled to prepare contrasting RNA bulks. RNA libraries were prepared from 3 μg of RNA and sequenced on a Hiseq 2000 platform with 100 bp paired end reads (Illumina). Both RNA extraction and RNA-Seq were conducted by Beijing Novogene Bioinformatics Technology Co. Ltd. The implementation of SNP calling and filtration and the identification of SNPs associated with the target trait were performed as described in Wang et al. [33].

2.8. SSR genotyping

Wheat simple sequence repeat (SSR) markers [35] (GWM, WMC, BARC, CFD, and GDM series; http://wheat.pw.usda.gov) on homoeologous group 2 chromosomes were screened for polymorphisms between ND399 and DD399. The polymorphic markers were used to genotype the F_2 population. PCR were performed on total volumes of 10 μL using an ABI9700 analyzer and the products were separated in 8% non-denaturing polyacrylamide gels as described by Li et al. [36] and visualized by silver staining.

2.9. Polymorphic SSR and STS markers development

The sequences of polymorphic SNPs detected by 90 K SNP array and BSR-Seq were used as queries in BLAST searches of CS chromosome sorting survey sequences (https://urgi.versailles.inra.fr/blast/blast.php) and whole genome assembly (IWGSC WGS v1, NRGene DeNovoMAGIC), and the homologous contigs were used to screen SSR motifs via BatchPrimer3 [37] or as templates to design sequence-tagged site (STS) markers (http://bioinfo.ut.ee/primer3-0.4.0/) with the following parameters: amplification product size of 200–600 bp, primer length of 18–24 bp, T_m of 55–65 °C, and GC content of 40%–60%.

3. Results

3.1. Phenotypic characterization of the semi-dwarf mutant DD399

The plant height of DD399 in cropping seasons 2011–2012 and 2013–2014 at BJ and GY varied from 54 to 60 cm and 55–61 cm, and the plant height of ND399 ranged from 71 to 72 cm and 70.2–77 cm, respectively (Fig. 1A, B). The average plant height of DD399 was 15 cm shorter than ND399. There were significant differences in TGW, peduncle length and internode lengths between DD399 and ND399 at both sites, except for internode 4 at BJ (Fig. 1A, B).

3.2. Gibberellic acid 3 (GA_3) response

Seeding and coleoptile length responses of ND399, DD399, CS, and Aibian 1 to exogenous GA_3 treatment were evaluated on 10-day old seedlings treated with 50 mg L^{-1} GA_3 solution after germination. The average seeding and coleoptile lengths of CS to such treatment were significantly higher than the non-treated control while no significant change was observed on DD399 and ND399 (Table 1).

3.3. Cell dimensions

The transverse and longitudinal sections of fully elongated peduncles in ND399 and DD399 at flowering were observed. The number of vascular bundles of ND399 was around 30 compared to about 26 for DD399. The arrangement of vascular bundles in ND399 appeared to be loose (Fig. 2A and B) throughout the transverse section. The longitudinal sections of ND399 and DD399 were also compared to search for cytological evidence of plant height reduction. Peduncle tissues of DD399 were composed of shorter cells than those of ND399 (Fig. 2C and D), indicating the likely cause of reduced plant height.

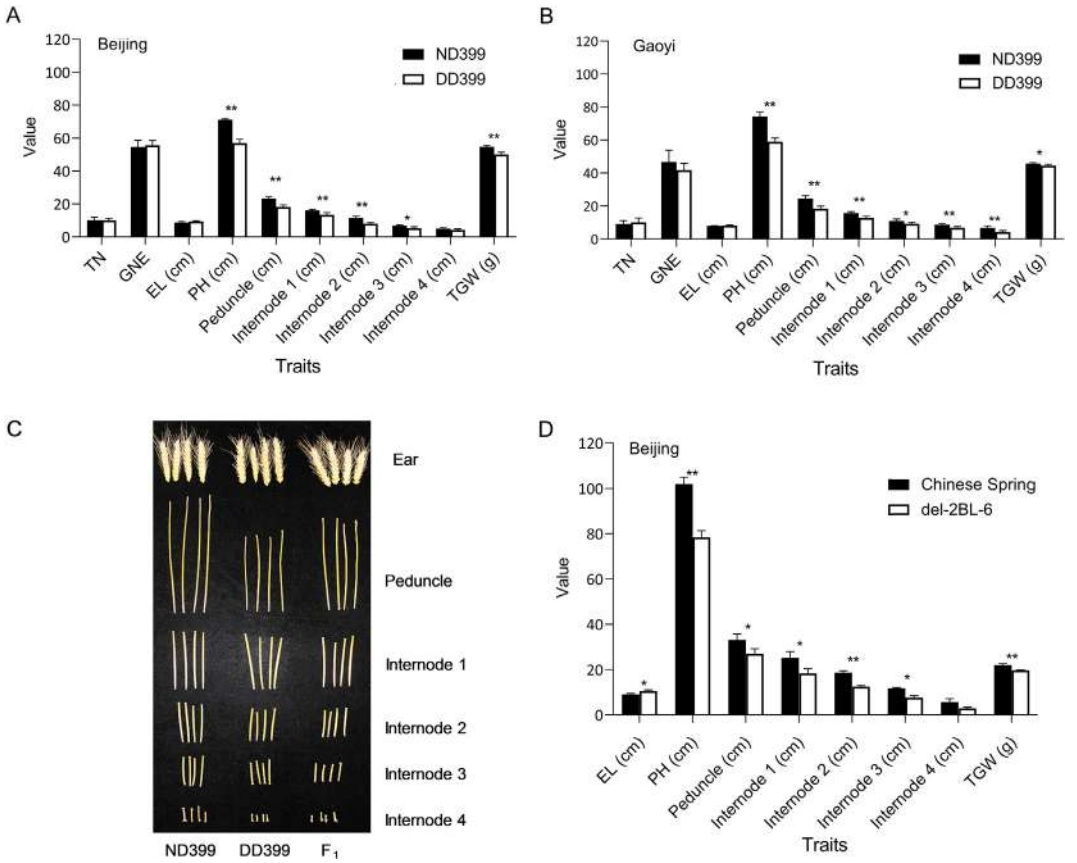

Fig. 1. Comparison of agronomic traits in ND300 and DD399. (A) and (B) Traits measures for ND399 and DD399 in 2014 at Beijing (A) and Gaoyi (B), respectively. (C) Heights of ND399, DD399, and the F_1. (D) Comparison of trait measures for Chinese Spring and Chinese Spring deletion line 2BL6-0.89–1.00 in 2014 at Beijing. *P*-values are from one-way ANOVA. ** and *, significantly different at $P < 0.01$ and $P < 0.05$, respectively. TN, tiller number; GN, grain number of the main spike; SL, spike length of the main spike; PH, plant height; TGW, thousand grain weight.

Table 1
Effects of GA_3 on seedling and coleoptile lengths of 10-day-old wheat plants.

Tissue	Line	0 mg L^{-1} GA_3	50 mg L^{-1} GA_3	*P*-value
Seedling	Chinese Spring	22.56 ± 1.40	32.88 ± 3.22	2.39e-12**
	DD399	16.03 ± 0.62	16.13 ± 0.72	0.72
	ND399	17.02 ± 1.11	17.38 ± 0.73	0.42
	Aibian 1	6.99 ± 0.29	7.02 ± 0.21	0.83
Coleoptile	Chinese Spring	4.95 ± 0.40	6.25 ± 0.66	7.23e-07**
	DD399	3.55 ± 0.21	3.40 ± 0.14	0.06
	ND399	3.28 ± 0.21	3.22 ± 0.19	0.52
	Aibian 1	2.15 ± 0.14	2.18 ± 0.13	0.66

*, $P < 0.05$; **, $P < 0.01$.

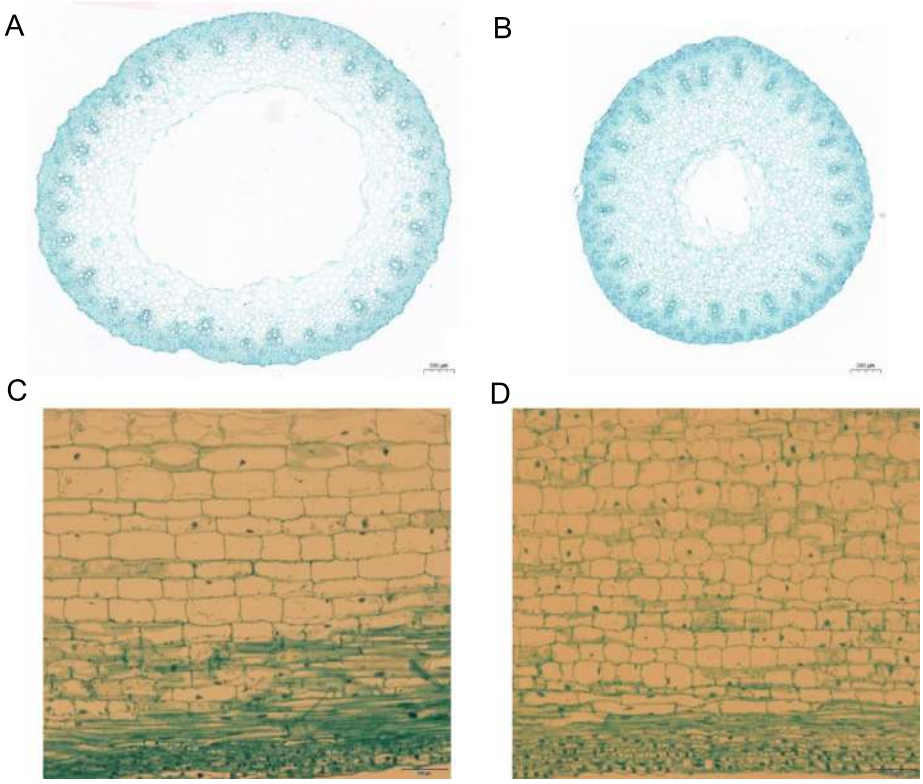

Fig. 2. Transverse and longitudinal culm sections from ND399 and DD399 at flowering. Anatomical structures in ND399 (A) and DD399 (B). Bar, 200 μm. Microstructure of parenchyma cells in the peduncles of ND399 (C) and DD399 (D). Bar, 100 μm.

3.4. Genetic analysis and mapping of the dwarf mutation

The height of the ND399 × DD399 F_1 (62–65 cm) was intermediate between the parents (Fig. 1C). The heights of the 2292 F_2 population were continuously distributed or unequally bimodal (Fig. 3A). The $F_{2:3}$ families segregated 354 homozygous short (45–59 cm): 1045 segregating: 893 homozygous tall (60–80 cm) ($\chi^2_{1:2:1}$ = 271.31, $P_{2df} < 0.001$). The distribution was clearly highly skewed in favor of tall plants (Fig. 3B).

Illumina 90 K iSelect SNP genotyping was applied to detect genetic differences between DD399 and ND399. Of the 81,587 potential SNPs arrayed in the chip, only 538 (0.66%) were polymorphic providing supportive evidence that DD399 was a spontaneous mutant of ND399. Three hundred and seventy one of the 538 SNPs could be mapped to 19 wheat chromosomes excluding 4D and 5D (Fig. 4A); 279 (75%) were located on homeologous group 2, with the majority (187/279 = 67%) located on chromosome 2B (Fig. 4A). These results indicated that a reduced height gene, tentatively designated *RhtDD399*, was located on chromosome 2B.

RNA-Seq analysis in 100 bp paired-end mode generated 74,744,011 and 79,234,419 clean reads for the tall and short bulks, respectively. With filtration criteria of allele frequency difference (AFD) > 0.8 and Fisher Exact Test *P*-value < 1e-10 313 SNPs were identified as putatively associated with plant height. Among them, 286 (91.37%) were located on chromosome 2BL (Fig. 4B), indicating that *RhtDD399* was located on 2BL. This result was consistent with the 90 K SNP chip results.

Only two (*Xwmc317* and *Xwmc361*) of the 160 SSR markers distributed on homoeologous group 2 chromosomes were polymorphic between ND399 and DD399 (Fig. 5A and B). Twenty two molecular markers linked with *Rht8* on chromosome 2D [38] were also tested but yielded none polymorphism between ND399 and DD399. Genotyping of *Xwmc317* and *Xwmc361* on 90 extremely tall and 90 extremely short F_2 plants revealed that the target DNA bands were present only in the tall plants as in ND399, and no products were produced by the short plants. In addition, the CS deletion line del-2BL6-0.89-1.00 was selected to determine the physical bin location of *Xwmc317* and *Xwmc361*. Target bands of

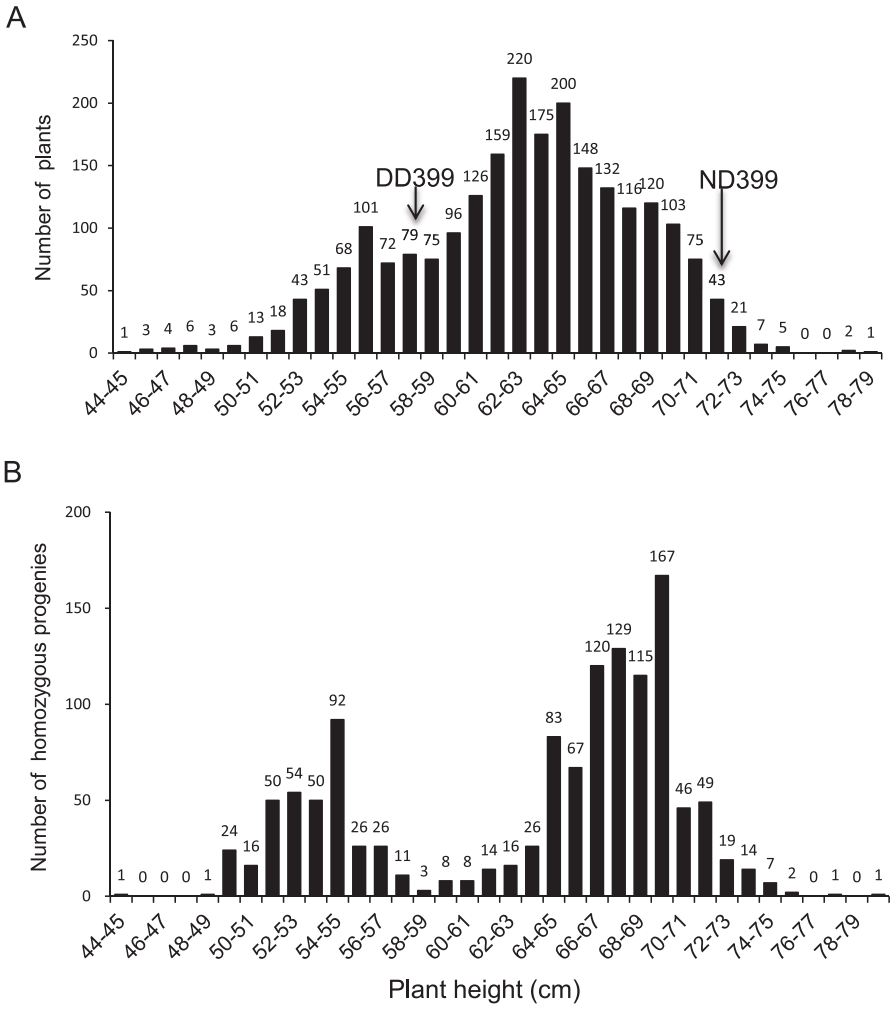

Fig. 3. Distribution of plant height in the segregating F_2 population (n = 2292) (A) and homozygous $F_{2:3}$ families (n = 1247) (B).

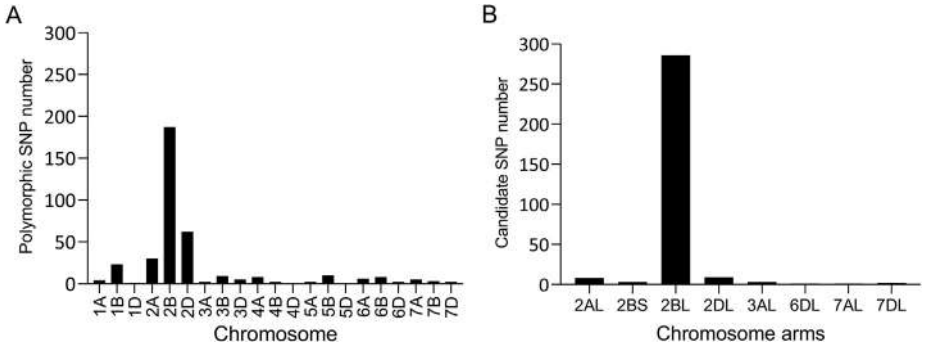

Fig. 4. Distribution of polymorphic SNPs on wheat chromosomes. (A) SNPs detected by the Illumina iSelect 90 K SNP assay. (B) SNPs detected by BSR-Seq analysis DNA bulks from extreme tall and short F_2 plants.

these two markers were also detected in CS but not in deletion line CS del-2BL6-0.89-1.00, indicating that *RhtDD399* was located in distal bin 2BL6-0.89-1.00.

3.5. Polymorphic marker development

The flanking sequences of *RhtDD399*-associated SNPs identified from the Illumina 90 K SNP chip assay and BSR-Seq analysis were used to blast the CS contigs and whole genome assembly to find homologous contigs for use in PCR primer design. Eight new SSR and seven STS markers polymorphic between ND399 and DD399 were developed (Table 2; Fig. 5C–E). All were dominant with bands amplified only in ND399 and not in the mutant. Genotyping of these markers on the 90-F_2 plant extreme phenotype groups also showed amplification by the tall plants as with ND399, but not the short plants, consistent with the results for SSR markers *Xwmc317* and *Xwmc361*. In addition, all these markers were located in the CS distal bin 2BL-0.89-1.00.

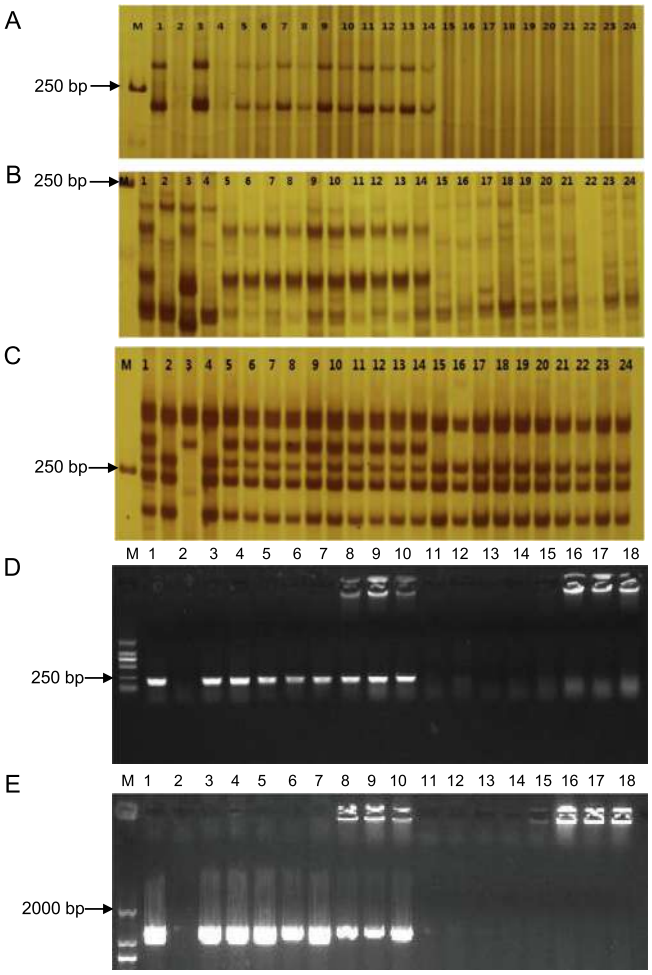

Fig. 5. PCR amplification of polymorphic markers. (A–C) Amplifications for markers *Xwmc317*, *Xwmc361*, and *Xwggc1605* in non-denaturing polyacrylamide gel electrophoresis (PAGE), respectively. 1, ND399; 2, DD399; 3, Chinese Spring; 4, Chinese Spring del2BL6-0.89–1.00; 5–14, tall F_2 plants; 15–24, short F_2 plants. (D and E) Amplification of STS markers *Xdd8* and *Xfh17*. 1, ND399; 2, DD399; 3–10, tall F_2 plants; 11–18, short F_2 plants. Arrows indicate polymorphic DNA bands. M, DNA Ladder 2000.

3.6. Deletion of a distal 2BL chromosome fragment in DD399

Based on the physical positions of the polymorphic markers on the CS 2BL reference sequence, four markers *Xwggc1596*, *Xwggc5434*, *Xwmc317*, and *Xwggc5801* were selected to genotype all 2292 F_2 plants. Target DNA bands were successfully amplified in the 1045 heterozygous (segregating $F_{2:3}$ families) and 893 homozygous tall plants (homozygous $F_{2:3}$ families), but were absent in the 354 homozygous short lines (homozygous $F_{2:3}$ families). All 17 dominant markers co-segregated with the tallness allele of *RhtDD399* even though they were dispersed across a 36 Mb (763 to 799 Mb) range in CS chromosome 2BL (Fig. 6; Table 2).

Comparison of the plant heights of CS and CS del-2BL6-0.89–1.00 also indicated a 24 cm difference (Fig. 1D). Significant differences were also observed in peduncle length, the 1st–3rd internode lengths and TGW (Fig. 1D). The consistent effects of CS del-2BL6-0.89-1.00 and DD399 on plant height and related traits, suggested a distal deletion of the chromosome 2BL in DD399.

4. Discussion

4.1. Relationship between RhtDD399 and known Rht genes

Two types of *Rht* genes have been described in wheat. Some, such as *Rht1* (*Rht-B1b*), *Rht2* (*Rht-D1b*), *Rht3* (*Rht-B1c*), and *Rht10* (*Rht-D1c*), were identified in natural populations and others, such as *Rht4*, *Rht5*, *Rht12*, and *Rht23*, were obtained by artificial mutagenesis [13]. Pleiotropism has been found for many *Rht* genes. For example, *Rht4*, *Rht5*, *Rht6*, *Rht7*, *Rht23*, *Rht24*, and *Rht25* showed negative impacts on agronomic traits whereas *Rht24* showed variable effects in different genetic backgrounds [14–16]. DD399 was a spontaneous mutant of cultivar ND399 with a 15 cm reduction in plant height. There was no significant difference in tiller number, panicle grain number and panicle length between ND399 and DD399, however, there was a significant decrease in 1000-grain weight relative to ND399.

Among the known reduced height genes *Rht4* obtained following radiation was located on chromosome 2BL [39]. Like *RhtDD399*, *Rht4* was reported to be linked with SSR marker *Xwmc317* in a doubled haploid population, accounting for up to a 15.4 cm height difference relative to its wild type [40]. Given that radiation predominantly causes deletions, it is likely that mutant alleles

Table 2
Polymorphic markers linked with *RhtDD399*.

Marker	Type	Forward primer (5′–3′)	Reverse primer (5′–3′)	Physical position on Chinese Spring 2BL	
				Start	End
Xdd8	STS	ATTTGCCATCAACTCAGTAAA	CTTAACAAACATGAAGGGATG	763,655,024	763,654,741
WGGC5434	STS	CTTGGGACCGTTTAGGTTGA	AATCCCTCCACCAATCACAA	767,129,685	767,130,189
WGGC5573	STS	TTTGTCGCATGGAGAGATGA	CAGATTGCACGCTATCCTGA	772,026,298	772,026,799
WGGC1558	SSR	CACAACGAATTCAGACGAT	CATGATTGAACTAGTGCTCA	777,526,549	777,526,700
Xwmc361	SSR	AATGAAGATGCAAATCGACGGC	ATTCTCGCACTGAAAACAGGGG	779,339,482	779,339,263
WGGC5764	STS	GCTTGTTCACAAATTCCACA	TTTTTGTCGGTTTTCTCTGG	780,777,788	780,777,366
WGGC3776	SSR	AATGGATGAATTCGGTCGAG	GGGTGTCATCTGGAGGAAAA	782,152,778	782,153,066
WGGC1565	SSR	GGATGACTGGAAGAGGCTA	AATCAGTGTGCCATTAGGG	782,958,759	782,958,604
Xwmc317	SSR	TGCTAGCAATGCTCCGGGTAAC	TCACGAAACCTTTTCCTCCTCC	783,892,317	783,892,175
Xfh7	STS	TGGCAGAAAACACACACCAT	CTCGAGGGAGGTGCTGATAG	790,441,580	790,442,915
WGGC1605	SSR	GGATGAGCGAAGAGGACT	CTCCTCCTCACCTATCTCG	790,584,050	790,584,203
WGGC5801	SSR	CAGCCTCAACGACCACAAC	AGACGTTCCTGACCATCCTCT	796,600,408	796,600,092
WGGC1596	SSR	AGGACGCCCTTCCTATTA	TTGCTTGTCACAATCCACT	797,325,945	797,326,100
WGGC1540	SSR	CGAACAACTCAGCTTCAGA	ATATCCTCTCGTCCTGCAC	797,339,882	797,339,726
WGGC5762	SSR	CGTAAGCAAAGAACAGCCAAG	GGCTGAGGAGAAGAAGCATTT	798,132,155	798,131,853
Xfh17	STS	TTCCCTAATTCCCAGGCTCT	CGGGTCTACTCTGCTTCTGG	798,134,530	798,135,771
BY47	STS	GAGGAGCTGGTGGGGTACTA	GTAATAGCCGCCCAAATCCG	799,029,300	799,030,010

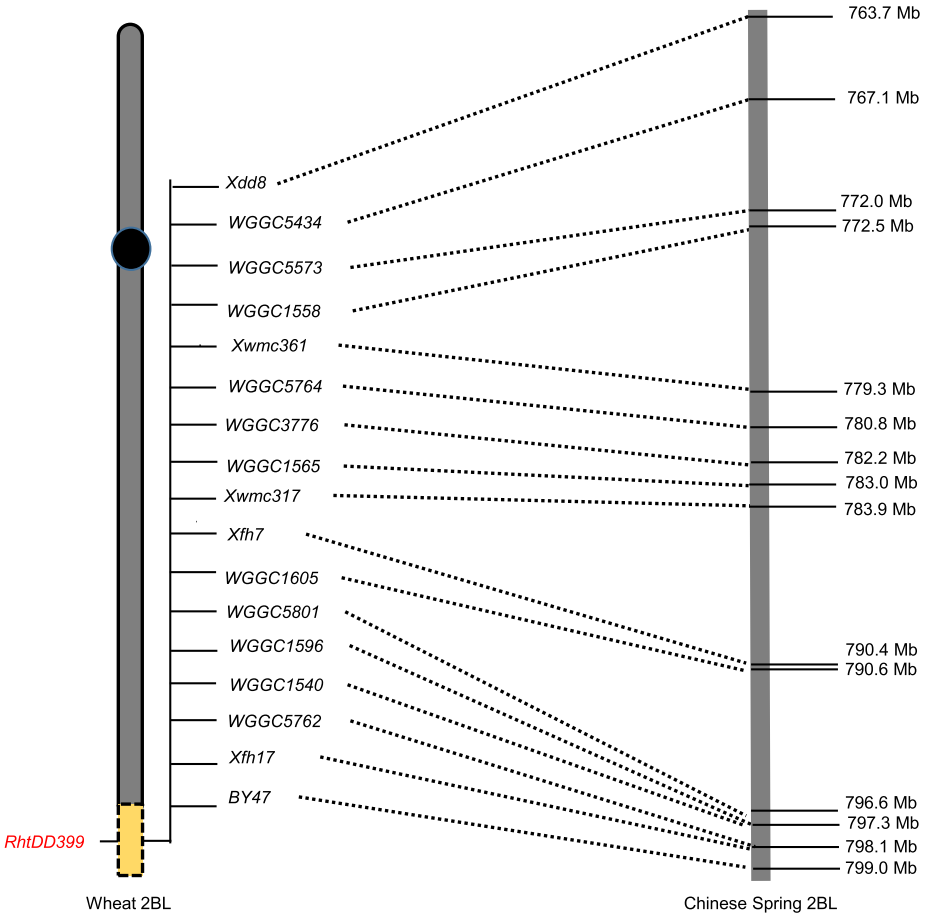

Fig. 6. Genetic (left) and physical (right) maps of *RhtDD399*. The physical positions of molecular markers at the far right of the physical map are according to the Chinese Spring reference sequence (http://www.wheatgenome.org/).

RhtDD399 and *Rht4* involve the same locus. Tests of allelism, genetic fine-mapping, and cloning of *Rht4* are required to clarify this situation.

4.2. Deletion of the chromosome segment led to reduced plant height and distorted segregation

Deletions of large chromosomal fragments of wheat chromosomes have significant impacts on agronomic traits. Ma et al. [41] found that some CS ditelosomic lines showed variations in response to Fusarium head blight (FHB) caused by *Fusarium* species, suggesting that the missing chromosome arms carried genes affecting FHB response. A similar phenomenon was also observed by Garvin et al. [42] whereby loss of a large chromosome 3DL fragment in cultivar Apogee led to enhanced FHB resistance. Sun et al. [43] mapped *Rht12*, a dominant GA-responsive reduced height gene in a gamma ray-induced wheat mutant that corresponded to a 11.21 Mb physical region at the terminal end of CS chromosome 5AL. They further found the reduced height was associated with deletion of a 10.73 Mb fragment of the distal region of 5AL. In the present study, both the Illumina iSelect 90 K SNP chip and BSR-Seq analyses revealed the absence of 17 molecular markers in the *RhtDD399* mutant relative to the wild type. Based on the complete association of all 17 markers with the tall phenotype and their physical locations it was evident that the mutant had a 36 Mb deletion that led to reduced height. Reduced plant height and TGW of CS deletion line del-2BL6-0.89–1.00 provided further evidence that a gene(s) involved in plant height was located in the distal region of chromosome 2BL.

Distorted segregation, that is, deviation from expected Mendelian segregation ratios has been attributed to competition between gametes or abortion of gametes and/or zygotes in animals [44]. Distorted segregation caused by differential transmission of gametes [45] in wheat aneuploid stocks [46] was the basis of gene location prior to molecular markers. Distorted inheritance of stem rust resistance gene *Sr11* was observed in the cross of Timstein and CS (ratio about 2.4:1), whereas a normal (ratio about 3:1) segregation was found in the crosses of Timstein with other varieties [47,48]. A pollen-killing gene linked to *Sr11* was proposed to be the reason of the differential transmission of gametes [45,47,48]. Similar situation was also observed in favor of these alleles (ratio about 4:1) that derived from *T. timopheevii* in the case of *Sr36*/*Pm6* transmission [49]. Peng et al. [50] and Li et al. [36] reported that gametes carrying certain alleles from Langdon durum had stronger vigor and higher competition ability than those alleles from wild emmer. Miao et al. [51] reported that genetic factor(s) in the centromere region caused distorted segregation of linked genetic markers on wheat chromosome 1B. In our study, it was evident that flower numbers of homozygous short plants in a segregating population were due to lower transmission of gametes carrying the deleted 2B chromosome. Such effects occur through competition of normal and deficient male gametes, whereas female transmission is usually normal.

4.3. Comparison of SNP chip and BSR-Seq in mapping RhtDD399

Both SNP-based pool genotyping and BSR-Seq have been used to map wheat genes. Both methods allow rapid identification of

SNPs associated with traits of interest. For example, SNP arrays were used for identification of stripe rust resistance genes *Yr26* [52], *Qyr.nwafu-6BL* [30], *QYrcen.nwafu-7BL* [53], *QYr.nwafu-3BS.2* and *QYr.nwafu-7BL* [54], leaf rust resistance QTL *QLr.hebau-1BL*, *QLr.bebau-2AS*, and *QLr.hebau-2DS* [55], and powdery mildew resistance genes in 36 wheat lines [31]. BSR-Seq provided an alternative approach for mapping stripe rust resistance genes *YrZh22* [33] and *Yr17* [56], early leaf senescence gene *els1* [57], and powdery mildew resistance genes *Pm4b* [58], *Pm61* [59], and *PmQ* [60]. In this study, Illumina 90 K SNP and BSR-Seq assays were both applied for mapping *RhtDD399* and both associated reduced height with the terminal region of chromosome 2BL. The very low frequency of SNPs (538/81,587 = 0.66 %) between ND399 and DD399 confirmed that DD399 was a spontaneous reduced height mutant in cultivar ND399. About one-half of the polymorphic SNPs (279/538 = 51.86%) were located on homoeologous group 2 chromosomes and 187 (187/279 = 67%) of those were located at the distal end of 2BL (Fig. 4A). BSR-Seq analysis identified 313 SNPs associated with *RhtDD399* between the two contrasting RNA bulks; 97.76% were located on homoeologous group 2 chromosomes and 86.26% of those were in the distal end of 2BL (Fig. 4B). It seems that BSR-Seq was more efficient than the Illumina 90 K SNP assay in identifying SNPs associated with the target trait.

Validation of the SNP markers in the segregating population revealed PCR amplification only in ND399 but not in DD399 and all of these dominant markers co-segregated with reduced height. The results indicated that these SNPs associated with the chromosome segment deletion identified by Illumina SNP assay and BSR-Seq analysis were not SNPs from the 2BL allelic DNA sequences or transcripts of ND399 and DD399 (null alleles), but from the DNA sequences or transcripts of ND399 2BL and ND399 or DD399 2AL, 2DL homoeolgs, and other homologs. High sequence identities among homeologs and homologs are major causes of false SNP calling in polyploid wheat. The unusually high frequencies of SNPs identified for chromosomes 2A and 2D compared to other background chromosomes (Fig. 4) likely reflected sequence polymorphisms between homoeologous chromosomes detected at reduced stringency in the absence of a homologous 2BL sequence.

5. Conclusions

We determined that reduced height in spontaneous dwarf mutant DD399 was caused by deletion of a 36 Mb terminal segment of chromosome 2BL. The predicted locus controlling plant height in the deleted region might be the same as *Rht4*.

CRediT authorship contribution statement

Qiuhong Wu, Yongxing Chen, and Zhiyong Liu conceived and designed the experiments. **Jingzhong Xie and Lingli Dong** performed bioinformatics analysis. **Qiuhong Wu, Yongxing Chen, Zhenzhong Wang, Ping Lu, and Yan Zhang** performed experiment and analyzed the data. **Rongge Wang, Chengguo Yuan, and Zhiyong Liu** developed the DD399 mutant. **Qiuhong Wu and Zhiyong Liu** wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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