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The Carotenoid Cleavage Dioxygenase Gene *CCD7-B*, at Large, Is Associated with Tillering in Common Wheat

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Abstract: Wheat, an important cereal crop, is responsible for the livelihoods of many people, and a component of national food security. Tillering, which determines plant architecture and spike number, is a critical agronomic trait of wheat. The carotenoid cleavage dioxygenase 7 (CCD7) has an important effect on the growth of tillers or lateral branches and lateral roots of plants. In order to study the relationship between *CCD7* and tillering in wheat, *CCD7-B* was isolated from 10 Chinese wheat varieties with different tiller numbers. Subsequently, bioinformatics, allelic variation analysis, and field experiments were performed. Wheat *CCD7-B* belongs to the retinal pigment epithelial membrane receptor (RPE65) superfamily; it displays the greatest homology with monocot CCD7 proteins. Phylogenetic analysis of wheat *CCD7-B* proteins indicated division into dicotyledonous and monocotyledonous clades. Allelic variation analysis of *CCD7-B* via *Srg*AI enzyme digestion (a marker of cleaved amplified polymorphic sequences) suggested that 262 Chinese wheat micro-core collections and 121 Chinese wheat major cultivars from the Yellow and Huai River Valley winter wheat region can be divided into two groups: *CCD7-B1* (C/T/T) and *CCD7-B2* (G/C/A). *CCD7-B1* showed better allelic variation than did *CCD7-B2* for increasing the number of effective tillers of wheat varieties in China. This study provides reference data for the application of *CCD7-B* alleles to wheat breeding and supports further research regarding the mechanism of tillering in common wheat.

Keywords: wheat; *CCD7-B*; CAPS marker; allele; tillering

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1. Introduction

Bread wheat (*Triticum aestivum* L., 2n = 6× = 42) is one of the most important cereal crops worldwide [1], along with maize (*Zea mays* L.) and rice (*Oryza sativa* L.) [2]; it is cultivated on 222 million hectares globally [3]. Wheat is a major staple food crop used by more than 30% of the world's population [4]. In 2050, the global population is expected to be 9 billion based on the current growth rate [5], highlighting a future threat global food security [6]. Therefore, an improvement in wheat yield potential is urgently needed. Many physiological and agronomic traits affect the final yield of wheat; these traits include the number of spikes per unit area, number of grains per spike, thousand grain weight, chlorophyll content, photosynthetic rate, and water-soluble carbohydrates. Among these

traits, tillering or shoot branching, which defines plant architecture and spike number, is considered a major determinant of wheat grain yield [1].

The process of tillering (i.e., shoot branching) is regulated by complex interactions among a wide range of hormonal, environmental, and developmental factors [7]. Studies of a series of highly tillered mutants including *more axillary growth* (*max*) in *Arabidopsis thaliana* [8–11], *dwarf* (*d*)/*high tillering dwarf* (*htd*) in *O. sativa* [12–17], *ramosus* (*rms*) in *Pisum sativum* [8,18–20], and *decreased apical dominance* (*dad*) in hybrid *Petunia* [21–25], revealed that carotenoid-derived branching could inhibit the *MAX/RMS/D* pathway, thereby controlling the outgrowth of axillary buds in higher plants [26–29]. Strigolactones (SLs), a novel class of plant hormones that determine the structures of plants [30,31], are among the products of the *MAX/RMS/D* pathway [15,32–35]. Several key genes associated with SLs have been identified; these include *D27* [1,15], *MAX3* [9], *MAX4* [8], and *MAX1* [10,36] in their biosynthesis, and *MAX2* [11] and *AtD14* [37] in their signaling pathway [27,29,38]. Carotenoid cleavage dioxygenase 7 (*CCD7*), which is encoded by *MAX3*/*RMS5*/*HTD1*/*DAD3* and localized to the plastid, is a stereospecific enzyme that participates in SL biosynthesis [9,23,39,40]. *CCD7* cleaves 9-*cis*-β-carotene into 9-*cis*-β-apo-10′-carotenal, leading to the formation of carlactone, which is then catalyzed by the cytochrome P450 oxygenase MAX1 to yield SLs [10,33,41,42]. Thus, *CCD7* inhibits the outgrowth of axillary buds and suppresses tillering through the control of strigolactone biosynthesis [9,40,41,43]. As a critical enzyme in SL biosynthesis, *CCD7* has been studied in many plants, including *Arabidopsis* [9], pea (*P. sativum* L.) [39], rice [40], and *Petunia* sp. [23]; loss of the *CCD7* gene results in increased branching. Vogel et al. (2010) reported that *SlCCD7* antisense tomato (*Solanum lycopersicum* L.) lines exhibit greatly increased branching because of reduced SL levels [41]. Wang et al. (2020) reported that a partial loss-of-function allele of *HTD1* ($HTD1^{HZ}$), which encodes *CCD7*, is responsible for increasing the number of tillers and improving grain yield in rice [34]. Sun (2020) found that the tomato *ccd7* mutant obtained by CRISPR/cas9 gene editing had the phenotype of lateral branches increasing and plant dwarfing [44]. Galili et al. (2021) also reported that *CCD7M14* encodes a truncated protein that resulted in a typical SL-deficient phenotype, increased branching, and reduced plant height, and was highly resistant to both *Phelipanche aegyptiaca* and *Orobanche crenatain* in chickpea [45].

Genes involved in the biosynthesis or signal transduction pathway of SLs are required for the regulation of tillering. However, information about the key regulatory genes and the roles of such genes in tillering of wheat remains scarce. Recently, Zhao et al. (2020) identified three *TaD27* genes in wheat [1]; they demonstrated that *TaD27-B* has critical roles in the regulation of tiller number through participation in SL biosynthesis in wheat. To elucidate the mechanism of tiller regulation in wheat, we isolated the *CCD7-B* gene from 10 Chinese wheat varieties with different tiller numbers; we divided these *CCD7-B* genes into *CCD7-B1* (C/T/T) and *CCD7-B2* (G/C/A) through allelic variation analysis with *Srg*AI enzyme digestion. The field experiments demonstrated that *CCD7-B1* wheat plants had more tillers and *CCD7-B2* wheat plants had fewer tillers, suggesting that *CCD7-B* has a critical role in wheat tillering regulation and *CCD7-B1* markedly increases wheat tiller number. This study may provide reference information for the application of *CCD7-B1* alleles in wheat breeding.

2. Materials and Methods

2.1. Plant Materials

Ten Chinese wheat varieties, which could be subdivided into two types on the basis of tiller number, were used for cloning and analysis of the tillering gene *CCD7* (Table 1). In total, 262 Chinese wheat micro-core collections (MCC-CNs, kindly provided by Professor Xueyong Zhang of the Chinese Academy of Agriculture Sciences) [46], representing 1% of the national wheat collections but over 70% of the genetic diversity, were assessed; these collections included Chinese Spring (CS), 155 landraces, 89 Chinese-bred wheat cultivars, and 17 introduced foreign accessions. MCC-CNs were used for allelic variation analysis, and distribution and frequency analyses, in the 10 wheat-growing regions of China. Overall,

121 Chinese wheat major cultivars grown in the Yellow and Huai River Valley winter wheat region were used for analysis of allelic variations and fertile tiller numbers. Ten Chinese wheat varieties and 262 MCC-CNs were planted in a greenhouse at 23 °C under a 16 h light/8 h dark regime for 2 weeks. In addition, 121 Chinese wheat major cultivars from the Yellow and Huai River Valley winter wheat region were planted in the field in Zhengzhou, Zhumadian, and Shangqiu during 2015 and in Zhengzhou during 2016. The planting density of each plot was 25 cm row spacing and 3 cm plant spacing. The area of each plot was 15 m^2, with the length of 10 m and width of 1.5 m. Three replicates were used for each cultivar. The phenotypic observation of tiller number for each individual in 1 m^2 of each plot was recorded.

Table 1. Wheat varieties used in *CCD7* gene cloning.

No.	Wheat Variety	FT-2015	FT-2016	Average
C-1	Yuyuan 3	8.3	9.7	9
C-2	Baomai 3	6.9	7	7.0
C-3	Jimai 20	11.4	16.3	13.9
C-4	Reijina	11.2	15.7	13.5
C-5	Hang2399	14.9	15.5	15.2
C-6	Luyuan 212	6.3	7.9	7.1
C-7	Wen 12	9.4	8.1	8.8
C-8	Yanmai 98	9.3	6.5	7.9
C-9	Beijingyemaizi	22.2	14.2	18.2
C-10	Shuiyuan86	16.2	18	17.1

2.2. Cloning of CCD7 Gene

Genomic DNA was isolated through the cetyltrimethylammonium ammonium bromide method from young leaves [47]; its quality was checked using a Nanodrop spectrophotometer and 1% agarose gel. Subsequently, this DNA was used as template for polymerase chain reaction (PCR) amplification with *CCD7* gene primers (forward primer: 5′-TACAAACCACCACAAGGAACC-3′, reverse primer: 5′-TGCGATTTTTGCCATTCATTCAT-3′). DNA amplification was performed in a 20 µL PCR reaction containing 1.0 µL of each primer (10 µM), 10 µL 2× GC Buffer I, 0.2 µL LA Taq polymerase (5 U/µL, Takara, Japan), 0.8 µL dNTPs (10 mM), 1 µL template DNA (100 ng), and 6 µL ddH_2O. The PCR cycling protocol was: 5 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 54 °C, and 3 min at 72 °C, and then a final extension of 7 min at 72 °C. The PCR product was checked on a 1% agarose gel to confirm *CCD7* gene amplification. The purified PCR products and cloning vector pGEM-T Easy (Promega, Madison, WI, USA) were ligated and transformed into 50 µL of *Escherichia coli* competent cells using the heat shock method. After incubation on lysogeny broth medium at 37 °C overnight, colony PCR was performed to check for positive clones. Positive clones, identified on the basis of colony appearance and colony PCR, were sent to a commercial company for sequencing.

2.3. SgrAI Enzymatic Digestion of the CCD7 Gene

The enzymatic digestion reaction was prepared in a 20 µL volume containing 2.0 µL 1× CutSmart Buffer, 0.5 µL *Sgr*AI enzyme (New England Biolabs, Ipswich, MA, USA), 6.0 µL PCR products, and 11.5 µL ddH_2O. The amplified PCR products were incubated with *Sgr*AI enzyme in a water bath at 37 °C for 2–3 h for digestion, then resolved on a 1.5% agarose gel and visualized in a gel documentation system.

2.4. Bioinformatics Analysis

Available sequences for the *CCD7* gene were saved and aligned for analysis using DNASTAR Lasergene v7.1. The ExPASy ProtParam online analysis tool (http://web.expasy.org/protparam/, accessed on 10 December 2021) was utilized for analysis of the physicochemical properties of the amino acid sequence; the NCBI CDD online analysis

tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, accessed on 10 December 2021) was used to analyze the conserved domains of the amino acid sequence. Finally, the online program ClustalW (www.ebi.ac.uk/clustalw, accessed on 10 December 2021) was used for multiple alignment; MEGA 7 was used to generate a phylogenetic tree through the neighbor joining method with 1000 bootstrap replicates.

2.5. Statistical Analysis

Statistical analysis was conducted for the tiller number of wheat varieties grown in three locations of China during 2015 and 2016. Analysis of variance was used to test for differences in tiller number among three locations in China for 2 years (2015–2016) in SPSS software, version 17. Analysis of variance was conducted at a significance level of $p < 0.05$ to determine whether differences were present among treatment means.

3. Results

3.1. Sequence and Phylogenetic Analysis of CCD7-B

The genomic databases of wheat diploid ancestral species such as *T. urartu* (AA group), *Aegilops tauschii* (DD group), and common wheat Chinese Spring (AABBDD) were searched using the sequence of the cloned *CCD7* gene in rice; specific primers were designed from the homologous *CCD7* gene sequences obtained. The amplified PCR products of *CCD7* genes in 10 wheat varieties with different tiller numbers were isolated, cloned, and sent to a commercial company for sequencing. Sequence alignment using Lasergene software (Figure 1) showed that all obtained sequences were consistent with the wheat B genome, and thus the gene was named *CCD7-B*. Furthermore, the alignment revealed point mutations at three sites of *CCD7-B* in 10 wheat varieties, including G/C at 1410 bp, C/T at 2294 bp, and A/T at 2549 bp. The wheat varieties C-3, C-4, C-5, C-9, and C-10, which had bases of C/T/T at these three sites, had more tillers than did the wheat varieties C-1, C-2, C-6, C-7, and C-8, which had G/C/A bases at these sites. Therefore, we subclassified *CCD7-B* genotypes into *CCD7-B1* (C/T/T) and *CCD7-B2* (G/C/A).

The *CCD7-B* gene has an open reading frame of 1884 bp, encoding 627 amino acid residues with a molecular weight of 69.8 kDa and a theoretical isoelectric point of 9.4. Analysis of the conserved domain showed that the *CCD7-B* protein shares a conserved domain with the retinal pigment epithelial membrane receptor (RPE65) superfamily, which is a member of the carotenoid cleavage dioxygenase (CCD) protein family in mammals. Sequence alignment of *CCD7* proteins (Figure 2) showed that wheat *CCD7-B* is similar to the *CCD7* proteins of other monocotyledonous and dicotyledonous plants. *CCD7-B* showed the greatest homology, with homologs in *Triticum dicoccoides* (99%), *Aegilops tauschii* (96%), *Hordeum vulgare* (95%), *Brachypodium distachyon* (86%), rice and maize (77%), and *Sorghum bicolor* (75%). However, *CCD7-B* showed lower similarity with CCD7 proteins of dicotyledons (50–56%). Moreover, *CCD7-B* proteins have highly conserved residues, including three glutamic acid (Glu) and four histidine (His) residues, which determine the catalytic activity or substrate specificity of CCD proteins.

Phylogenetic analysis (Figure 3) showed that *CCD7* proteins of plants could be clearly divided into dicotyledons and monocotyledons. The *CCD7* proteins of dicot plant species such as apple (*Malus domestica* Borkh.), *A. thaliana*, and potato (*Solanum tuberosum* L.) were clustered in one clade, while the *CCD7* proteins of monocot plant species including *T. dicoccoides*, *A. tauschii*, *H. vulgare*, *B. distachyon*, rice, and maize were clustered with the *CCD7-B* proteins of wheat varieties. *CCD7-B* showed close evolutionary relationships with *T. dicoccoides*, *A. tauschii*, *H. vulgare*, and *B. distachyon*. In the monocot clade, *CCD7-B* proteins of the 10 cloned wheat varieties were subdivided into two categories, with C-3/4/5/9/10 in one class (the 609th amino acid residue of *CCD7-B1* is Val, corresponding to a 2549th nucleotide of T, Figure 1B) and C-1/2/6/7/8 in the other class (the 609th amino acid residue of *CCD7-B2* is Glu, corresponding to a 2549th nucleotide of A), consistent with the results of nucleotide-mutation analysis.

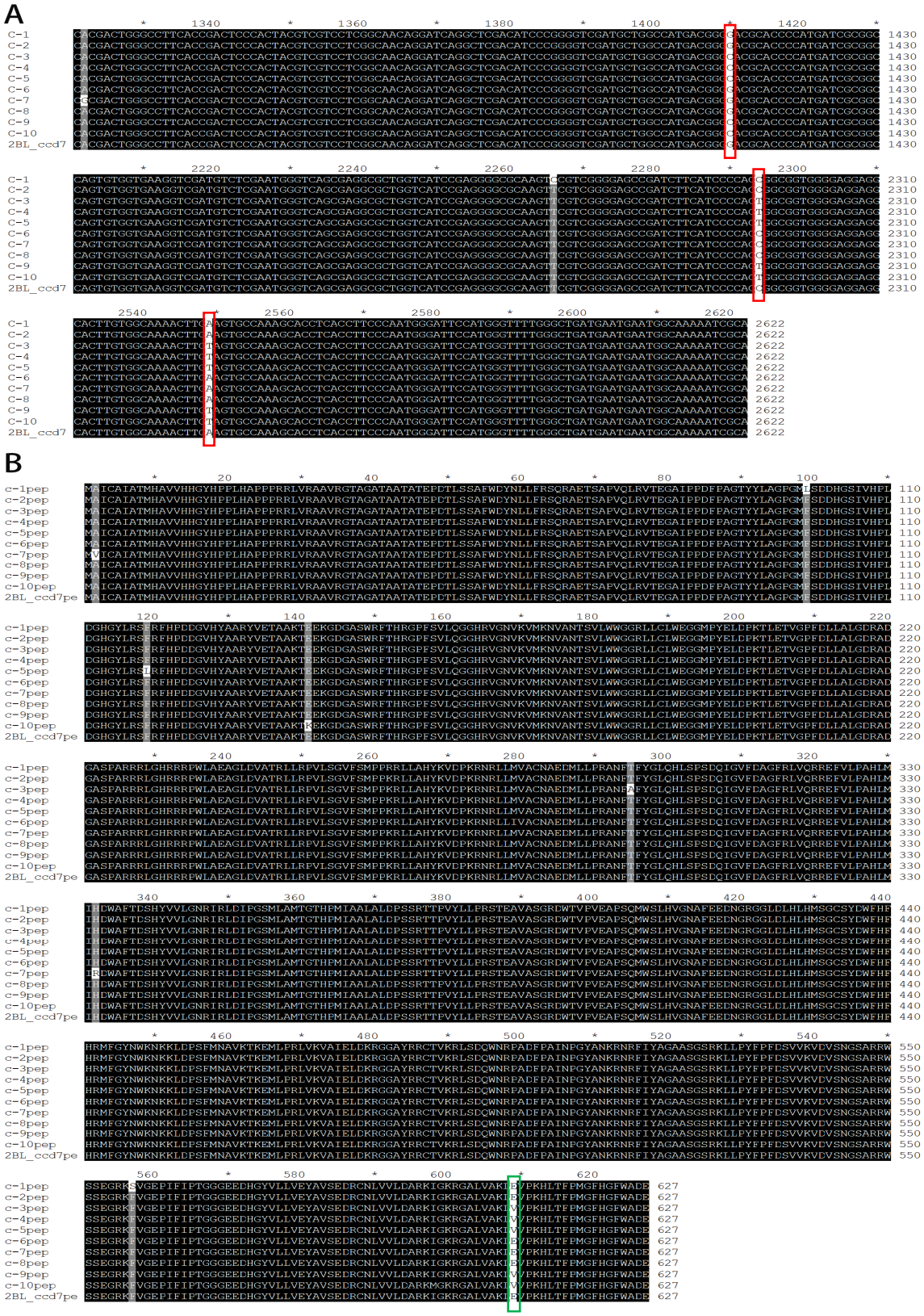

Figure 1. Multiple alignment of *CCD7* genes (**A**) and proteins (**B**) in different wheat varieties. The alignment was generated in the Lasergene software package MegAlign Version 7. The red boxes indicate the three sites of point mutation within the cloned wheat sequences; the green box indicate the amino acid of the third mutation point in the cloned wheat sequences.

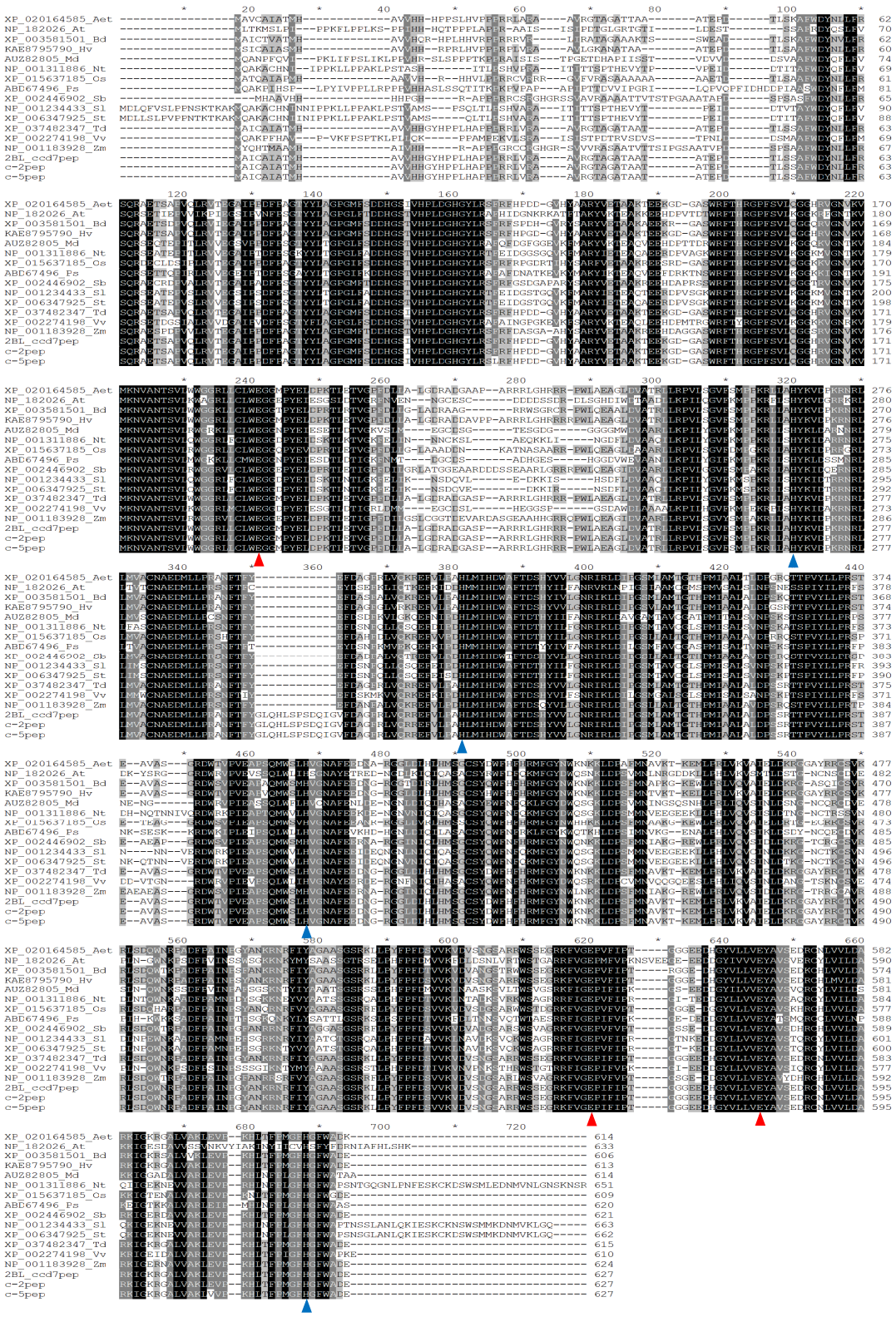

Figure 2. Multiple alignment of *CCD7* proteins in different monocots and dicots. The names of *CCD7* proteins are at the left and amino acid numbers are indicated at the right of the alignment. Red and blue triangles represent conserved iron-binding Glu and His residues, respectively. *Aet*, *Aegilops tauschii*, *At*, *Arabidopsis thaliana*, *Bd*, *Brachypodium distachyon*, *Hv*, *Hordeum vulgare*, *Md*, *Malus domestica*, *Nt*, *Nicotiana tabacum*, *Os*, *Oryza sativa*, *Ps*, *Pisum sativum*, *Sb*, *Sorghum bicolor*, *Sl*, *Solanum lycopersicum*, *St*, *Solanum tuberosum*, *Td*, *Triticum dicoccoides*, *Vv*, *Vitis vinifera*, *Zm*, *Zea mays*.

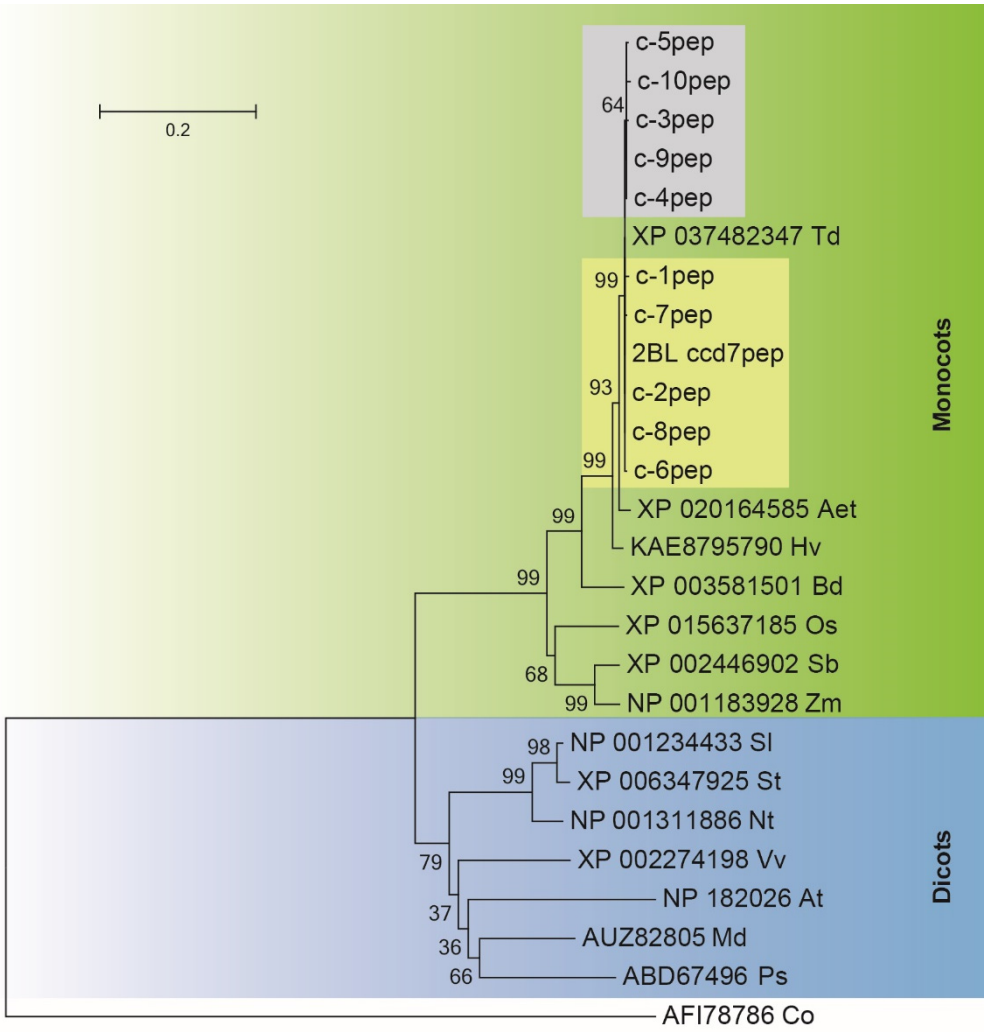

Figure 3. Phylogenetic analysis of *CCD7* proteins in different monocots and dicots. The deduced amino acid sequences of *CCD7* were aligned using the ClustalW (www.ebi.ac.uk/clustalw, accessed on 10 December 2021) online program. The phylogenetic tree was constructed using MEGA7 by the neighbor joining (NJ) method, with 1000 bootstrap replicates. The tree clusters in two groups, those demarcated by green and blue areas, into monocots and dicots, respectively. The grey and yellow squares indicate the two sub-classes of wheat *CCD7-B* proteins. *Co*, *Coleochaete orbicularis.*

3.2. Allelic Variation Analysis of the CCD7-B Gene with SgrAI Enzyme Digestion

Sequence analysis of the *CCD7-B* gene revealed two *Sgr*AI cleavage sites in *CCD7-B1* and three *Sgr*AI cleavage sites in *CCD7-B2*. To identify the genotypes of *CCD7-B* in different wheat varieties, 262 MCC-CNs were used for allelic variation analysis (Table S1). *Sgr*AI enzymatic digestion (Figure 4) and sequence analysis (Figure 5) of the amplified PCR products divided the MCC-CNs into two groups based on cleaved amplified polymorphic sequence (CAPS) markers: 176 MCC-CN wheat genotypes (67%) with cleavage results of 361/867/1396 bp were *CCD7-B1*, whereas 86 MCC-CN wheat genotypes (33%) with values of 336/361/867/1066 bp were *CCD7-B2*. The mean number of tillers of *CCD7-B1* wheat varieties was 9.6, while the mean number of tillers of *CCD7-B2* wheat varieties was 8.4, indicating that *CCD7-B1* was a better allelic variation than *CCD7-B2* for increasing the number of tillers among wheat varieties grown in China.

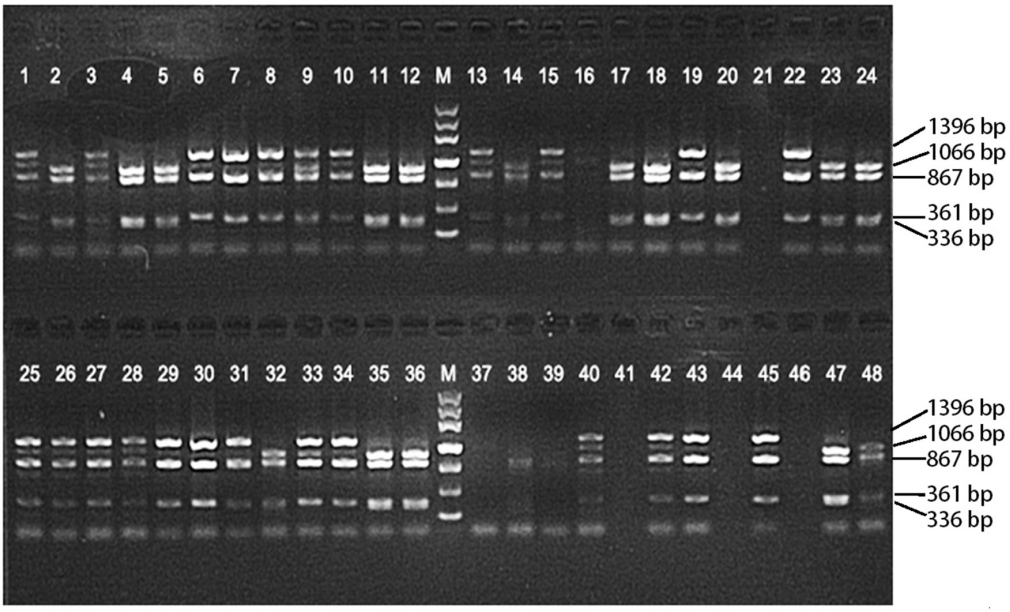

Figure 4. *CCD7-B* allelic variation for the less and more tiller number in Chinese wheat micro-core collections (MCC-CN). The names of MCC-CN wheat genotypes are given on the gel image: 1. Jinghong 5, 2. Neimai 11, 3. Jinchun 3, 4. Lianglaiyoubaipixiaomai, 5. Bihongsui, 6. Xiaobaimai, 7. Hongpixiaomai, 8. Dabaipi, 9. Xiaohongpi, 10. Dingxingzhai, 11. Honglidangnianlao, 12. Chunxiaomai, 13. Huoliaomai, 14. Dahongmai, 15. Shanxibaimai, 16. Niuzhijia, 17. Mahuaban, 18. Jiahongmai, 19. Hongjinmai, 20. Baiqimai, 21. Xiaokouhong, 22. Lanhuamai, 23. Daimanghongmai, 24. Zhuoludongmai, 25. Hongmai, 26. Honglaomai, 27. Youmangbaifu, 28. Hongpidongmai, 29. Xinshiwumang, 30. Youmangbaifu, 31. Baiqiumai, 32. Laomai, 33. Xiaobaimang, 34. Zhongyou 9507, 35. Jinmai 8, 36. Fengkang 2, 37. Changzhi 6406, 38. Beijing 8, 39. Yuandong 822, 40. Lvhan 328, 41. Yanan 11, 42. Nongda 183, 43. Nongda 311, 44. Nongda 139, 45. Mingxian 169, 46. Dongfanghong 3, 47. Xianmai, 48. Jiangxizao. M indicates DNA band size.

Figure 5. Sequencing and identification of CAPS loci of *CCD7-B* gene of wheat micro-core collections in China. The red box indicates the *Sgr*AI enzyme digestion site.

Furthermore, the distribution and frequencies of *CCD7-B1* and *B2* alleles in the MCC-CNs were studied among the 10 wheat-growing regions of China (Table 2). *CCD7-B1* and *CCD7-B2* both occurred in all 10 wheat-growing regions of China. *CCD7-B1* was widely distributed among the wheat varieties grown in the Yellow and Huai River Valley winter wheat region (16.79%) and Northern winter wheat region (8.78%), while *CCD7-B2* was widely distributed among the wheat varieties grown in the Middle and Low Yangtze valley winter wheat region (8.78%). The Northern winter wheat region (13/32, 40.6%) and Yellow and Huai River Valley winter wheat region (29/64, 45.3%) contained more improved wheat varieties (bred cultivars, Table S1), whereas the Middle and Low Yangtze valley winter wheat region (23/31, 74.2%) had more rural wheat varieties (landraces), indicating that *CCD7-B1* had been effectively selected during the breeding process.

Table 2. The distribution and frequencies of *CCD7-B* alleles in Chinese wheat micro-core collections.

Wheat Growing Regions of China	*CCD7-B1* (C/T/T)		*CCD7-B2* (G/C/A)	
	Varieties	Frequency (%)	Varieties	Frequency (%)
Northern spring wheat region	12	4.58	2	0.76
Northern winter wheat region	23	8.78	9	3.44
Northeastern spring wheat region	10	3.82	3	1.15
Southern winter wheat region	3	1.15	6	2.29
Yellow and Huai River Valley winter wheat region	44	16.79	20	7.63
Qing-Tibetan plateau spring wheat region	14	5.34	2	0.76
Northwestern spring wheat region	19	7.25	3	1.15
Southwestern winter wheat region	18	6.87	12	4.58
Xinjiang winter spring wheat region	12	4.58	2	0.76
Middle and Low Yangtze valley winter wheat region	8	3.05	23	8.78
Foreign varieties	13	4.96	4	1.53
Sum	176	67.18	86	32.82

3.3. Effects of Different Alleles of CCD7-B on Fertile Tiller Number

Because both *CCD7-B1* (16.79%) and *CCD7-B2* (7.63%) were widely distributed among the wheat varieties grown in the Yellow and Huai River Valley winter wheat region, 121 major Chinese wheat cultivars in this region were used for the analysis of *CCD7-B* allelic variation. Among them, 86 cultivars (71.1%) contained the *CCD-B1* haplotype and 35 cultivars (28.9%) contained the *CCD-B2* haplotype (Table S2), indicating that *CCD-B1* has been strengthened through breeding and is an excellent allelic variation.

To further explore the relationship between the *CCD-B1* gene and tillering, fertile tiller numbers of the 86 wheat cultivars with the *CCD-B1* haplotype were observed during 2015 in Zhengzhou, Zhumadian, and Shangqiu, and during 2016 in Zhengzhou (Table S2). The mean fertile tiller number was 9.5. In Zhengzhou (2015), Yanke 028 (6.7) produced the minimum number of tillers, while Xiaoyan 81 (14.1) produced the maximum number of tillers. At Zhumadian (2015) and Shangqiu (2015), the minimum number of tillers was produced by Xinmai 9 (6.2 and 6.3, respectively), and the maximum number was from Yumai 8 (13.2 and 13.2, respectively). In Zhengzhou (2016), the minimum number of tillers was observed in Anmai 8 (3.7), and the maximum number was observed in Shan 160 (13.2).

To verify the relationship between *CCD-B2* gene and tillering, the tiller numbers of the 35 wheat cultivars with the *CCD-B2* haplotype were observed during 2015 in Zhengzhou, Zhumadian, and Shangqiu and during 2016 in Zhengzhou (Table S2). Lankaoaizao 8 (5.6) had a significantly lower mean tiller number than did other wheat varieties, whereas the mean tiller number of Xinhan 1 (10.3) was significantly higher. During 2015 in Zhengzhou, Zhumadian, and Shangqiu, and 2016 in Zhengzhou, the minimum number of tillers was produced by Lankaoaizao 8 (6.4, 5.4, 5.4, and 4.9, respectively). In Zhengzhou (2015), the maximum number of tillers was produced by Jun9917 (12.9). In Zhumadian (2015) and

Shangqiu (2015), the maximum number of tillers was produced by Xinhan1 (10.3 and 10.2, respectively). In Zhengzhou (2016), the maximum number of tillers was obtained from Jimai 22 (11.0).

4. Discussion

SLs have an important role in shoot branching or tillering, the regulation of which is an essential determinant of plant architecture and grain yield in cereal crops [48]. As a stereospecific enzyme in SLs biosynthesis pathway, the use of *CCD7* may increase both wheat tiller and yield. Thus, research on sequence diversity of *CCD7* in wheat germplasm is essential to develop new potential genes for wheat breeding. However, studies of *CCD7* in wheat are particularly rare. To understand how the *CCD7* gene regulates tillering and how it has been utilized and selected during the breeding of Chinese wheat, *CCD7* genes were isolated from 10 wheat varieties with different tiller numbers in this study; obtained sequences that were consistent with the wheat B genome were designated *CCD7-B*. The *CCD7*s in 10 varieties showed different sequences in both nucleotide and protein level, indicating a rich variation in wheat germplasm. Phylogenetic analysis showed that CCD7 proteins in wheat were clustered with *CCD7* in *Triticum dicoccoides* in the monocotyledonous clade, separated from the dicotyledonous clade. Appiano et al. [49] reported that vulnerability proteins of monocots and dicots differed phylogenetically. Qin et al. [50] reported that wheat exhibits consistent phylogenetic relationships with other monocot species in the *CCD1* and *CCD4* genes. Colasuonno et al. [51] showed clear clustering of orthologs according to gene family, and maximum similarity with rice and *Brachyopodium* (monocot). These results are consistent with the findings of the present study.

To accelerate the efficient utilization of *CCD7-B* in modern wheat-breeding programs, molecular markers need to be developed for molecular-assisted selection. The CAPS marker, which is also known as the PCR-RFLP marker [52], has been frequently used for genotyping, map-based cloning, and molecular genetic studies in plants [53–55]. In the present study, only one convenient CAPS maker was developed that could distinguish all the *CCD7* sequences, which could accelerate the breeding progress with CCD7. Sequence analysis revealed two and three *Sgr*AI cleavage sites in *CCD7-B1* and *CCD7-B2*, respectively. *Sgr*AI enzymatic digestion results of the amplified PCR products from MCC-CN resources showed two groups based on the CAPS marker. In terms of molecular weight, among 262 MCC-CN wheat varieties, 176 (67%) varieties showed a pattern of 361/867/1396 bp, while 86 (33%) varieties showed a pattern of 336/361/867/1066 bp. The sequences of 40 amplified PCR products sent for sequencing also supported two groups based on nucleotide variations in MCC-CNs (Figure 5). *Sgr*AI enzyme digestion and sequence analysis both indicated sub-classification of the *CCD7-B* genotype into *CCD7-B1* (C/T/T) and *CCD7-B2* (G/C/A), showing that this CAPS marker can accurately distinguish point mutations in the *CCD7-B* gene.

Allelic variations can play essential roles in crop breeding. Breeders have successfully doubled grain yield over a few decades through the introduction of specific alleles of genes regulating gibberellin (GA) synthesis [56] or signaling [57] during the period known as the "Green Revolution". Wang et al. [34] reported that $HTD1^{HZ}$, which is a partial loss-of-function allele of *HTD1*, causes a significant increase in the grain yield of rice. To explore the roles of different alleles of *CCD7-B* in tillering, allelic variation analysis and field experiments were performed. The deduced *CCD7-B1* protein sequences of the wheat varieties C-3/4/5/9/10 differed from the *CCD7-B2* protein sequences of C-1/2/6/7/8; specifically, the 609th amino acid residue of *CCD7-B1* is Val, while the same amino acid residue of CCD7-B2 is Glu, but all 10 wheat varieties showed strong conservation of three glutamic (Glu) and four histidine (His) acid residues that determine the catalytic activity or substrate specificity of CCD proteins [23,41]. Thus, the 609th amino acid residue of *CCD7-B* may affect its protein functions or the promoter sequences may differ between *CCD7-B1* and *CCD7-B2*, allowing for specific regulation of *CCD7-B* expression; further investigation into the protein activities, gene expression, and transformation should be conducted to

clarify their functions. However, among the wheat varieties widely distributed in China, the *CCD7-B1* allele has been effectively selected in the breeding process. Furthermore, the high mean number of tillers indicated that *CCD7-B1* is a preferable allelic variation to *CCD7-B2*. The tiller number in wheat regulates plant canopy size, photosynthetic area, and the number of spikes bearing grains at maturity [58]. Sakamoto and Matsuoka [59] reported that the tiller number in rice is essential for grain yield; the number of tillers must be regulated to maximize the yield potential of the crop. Thus, the rational use of *CCD7-B* alleles to regulate the tiller number is a promising option to improve wheat yield. Because of the large and complex genome in the widely planted common wheat, the group A and D sequences of *CCD7* gene must be cloned and further studied.

Plants can maximize their utilization of light energy to increase economic effciency and crop yield [60]. In the 1960s and 1970s, global crop yields were increased to an unprecedented degree because of the extensive utilization of semi-dwarf cultivars [56,57]. However, reducing wheat plant height leads to compaction of the leaf layer and reduction in light energy utilization. Yield improvement requires the construction of an ideal plant form, for which intensive research has been conducted in rice [13,15,34,40], maize (Pan et al. 2016), and wheat (Zhao et al. 2020). *HTD1* and *Semidwarf 1* (*SD1*) have both contributed to the improvement in plant architecture in modern rice varieties since the "Green Revolution" [34]. More research on the mechanism of tillering regulated by *CCD7* in wheat should be arranged for the further utilization of *CCD7* in wheat breeding in future.

5. Conclusions

In this study, bioinformatics, allelic variation analysis, and field experiments were performed to identify the effects of the carotenoid cleavage dioxygenase gene *CCD7-B* on wheat tiller numbers. Wheat *CCD7-B* belongs to the retinal pigment epithelial membrane receptor (RPE65) superfamily; it displays the greatest homology with monocot *CCD7* proteins. Phylogenetic analysis of wheat *CCD7-B* proteins showed sharp division into dicotyledonous and monocotyledonous clades. Allelic variation analysis of *CCD7-B* via SrgAI enzyme digestion (a marker of cleaved amplified polymorphic sequences) suggested that 262 Chinese wheat micro-core collections and 121 major Chinese wheat cultivars from the Yellow and Huai River Valley winter wheat region can be divided into a *CCD7-B1* (C/T/T) group and a *CCD7-B2* (G/C/A) group. *CCD7-B1* was a more favorable allelic variation than *CCD7-B2* for increasing the number of effective tillers in wheat varieties grown in China. This study will promote the rational application of *CCD7-B* alleles in wheat breeding.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12020306/s1, Table S1. Fertile tillers of MCC wheat genotypes cultivated in different regions of China. Table S2. Fertile tillers of Chinese wheat main cultivars in Yellow and Huai River Valley Winter Wheat Region.

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References

1. Zhao, B.; Wu, T.T.; Ma, S.S.; Jiang, D.J.; Bie, X.M.; Sui, N.; Zhang, X.S.; Wang, F. *TaD27-B* gene controls the tiller number in hexaploid wheat. *Plant Biotechnol. J.* **2020**, *18*, 513–525. [CrossRef] [PubMed]
2. Tshikunde, N.M.; Mashilo, J.; Shimelis, H.; Odindo, A. Agronomic and physiological traits, and associated Quantitative Trait Loci (QTL) affecting yield response in wheat (*Triticum aestivum* L.), a review. *Front. Plant Sci.* **2019**, *10*, 1428. [CrossRef] [PubMed]
3. Mondal, S.; Rutkoski, J.E.; Velu, G.; Singh, P.K.; Crespo-Herrera, L.A.; Guzmán, C.; Bhavani, S.; Lan, C.; He, X.; Singh, R.P. Harnessing diversity in wheat to enhance grain yield, climate resilience, disease and insect pest resistance and nutrition through conventional and modern breeding approaches. *Front. Plant Sci.* **2016**, *7*, 991. [CrossRef] [PubMed]
4. Agharbaoui, Z.; Leclercq, M.; Remita, M.A.; Badawi, M.A.; Lord, E.; Houde, M.; Danyluk, J.; Diallo, A.B.; Sarhan, F. An integrative approach to identify hexaploid wheat miRNAome associated with development and tolerance to abiotic stress. *BMC Genom.* **2015**, *16*, 339. [CrossRef]
5. Deveshwar, P.; Prusty, A.; Sharma, S.; Tyagi, A.K. Phytohormone-mediated molecular mechanisms involving multiple genes and QTL govern grain number in rice. *Front. Genet.* **2020**, *11*, 586462. [CrossRef]
6. Nutan, K.K.; Rathore, R.S.; Tripathi, A.K.; Mishra, M.; Pareek, A.; Singla-Pareek, S.L. Integrating the dynamics of yield traits in rice in response to environmental changes. *J. Exp. Bot.* **2020**, *71*, 490–506. [CrossRef]
7. Kebrom, T.H.; Spielmeyer, W.; Finnegan, E.J. Grasses provide new insights into regulation of shoot branching. *Trends Plant Sci.* **2013**, *18*, 41–48. [CrossRef]
8. Sorefan, K.; Booker, J.; Haurogné, K.; Goussot, M.; Bainbridge, K.; Foo, E.; Chatfield, S.; Ward, S.; Beveridge, C.; Rameau, C.; et al. MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. *Genes Dev.* **2003**, *17*, 1469–1474. [CrossRef]
9. Booker, J.; Auldridge, M.; Wills, S.; McCarty, D.; Klee, H.; Leyser, O. MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. *Curr. Biol.* **2004**, *14*, 1232–1238. [CrossRef]
10. Booker, J.; Sieberer, T.; Wright, W.; Williamson, L.; Willett, B.; Stirnberg, P.; Turnbull, C.; Srinivasan, M.; Goddard, P.; Leyser, O. MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. *Dev. Cell* **2005**, *8*, 443–449. [CrossRef]
11. Stirnberg, P.; Furner, I.J.; Ottoline Leyser, H.M. MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. *Plant J.* **2007**, *50*, 80–94. [CrossRef] [PubMed]
12. Ishikawa, S.; Maekawa, M.; Arite, T.; Onishi, K.; Takamure, I.; Kyozuka, J. Suppression of tiller bud activity in tillering dwarf mutants of rice. *Plant Cell Physiol.* **2005**, *46*, 79–86. [CrossRef] [PubMed]
13. Arite, T.; Iwata, H.; Ohshima, K.; Maekawa, M.; Nakajima, M.; Kojima, M.; Sakakibara, H.; Kyozuka, J. DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. *Plant J.* **2007**, *51*, 1019–1029. [CrossRef] [PubMed]
14. Arite, T.; Umehara, M.; Ishikawa, S.; Hanada, A.; Maekawa, M.; Yamaguchi, S.; Kyozuka, J. d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant Cell Physiol.* **2009**, *50*, 1416–1424. [CrossRef]
15. Lin, H.; Wang, R.; Qian, Q.; Yan, M.; Meng, X.; Fu, Z.; Yan, C.; Jiang, B.; Su, Z.; Li, J.; et al. DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. *Plant Cell* **2009**, *21*, 1512–1525. [CrossRef]
16. Jiang, L.; Liu, X.; Xiong, G.; Liu, H.; Chen, F.; Wang, L.; Meng, X.; Liu, G.; Yu, H.; Yuan, Y.; et al. DWARF 53 acts as a repressor of strigolactone signalling in rice. *Nature* **2013**, *504*, 401–405. [CrossRef]
17. Zhou, F.; Lin, Q.; Zhu, L.; Ren, Y.; Zhou, K.; Shabek, N.; Wu, F.; Mao, H.; Dong, W.; Gan, L.; et al. D14-SCF^{D3}-dependent degradation of D53 regulates strigolactone signalling. *Nature* **2013**, *504*, 406–410. [CrossRef]
18. Beveridge, C.A.; Ross, J.J.; Murfet, I.C. Branching in Pea (Action of Genes Rms3 and Rms4). *Plant Physiol.* **1996**, *110*, 859–865. [CrossRef]
19. Morris, S.E.; Turnbull, C.G.; Murfet, I.C.; Beveridge, C.A. Mutational analysis of branching in pea. Evidence that Rms1 and Rms5 regulate the same novel signal. *Plant Physiol.* **2001**, *126*, 1205–1213. [CrossRef]
20. Foo, E.; Bullier, E.; Goussot, M.; Foucher, F.; Rameau, C.; Beveridge, C.A. The branching gene RAMOSUS1 mediates interactions among two novel signals and auxin in pea. *Plant Cell* **2005**, *17*, 464–474. [CrossRef]
21. Snowden, K.C.; Simkin, A.J.; Janssen, B.J.; Templeton, K.R.; Loucas, H.M.; Simons, J.L.; Karunairetnam, S.; Gleave, A.P.; Clark, D.G.; Klee, H.J. The Decreased apical dominance1/*Petunia hybrida* CAROTENOID CLEAVAGE DIOXYGENASE8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. *Plant Cell* **2005**, *17*, 746–759. [CrossRef] [PubMed]
22. Simons, J.L.; Napoli, C.A.; Janssen, B.J.; Plummer, K.M.; Snowden, K.C. Analysis of the DECREASED APICAL DOMINANCE genes of petunia in the control of axillary branching. *Plant Physiol.* **2007**, *143*, 697–706. [CrossRef] [PubMed]
23. Drummond, R.S.; Martínez-Sánchez, N.M.; Janssen, B.J.; Templeton, K.R.; Simons, J.L.; Quinn, B.D.; Karunairetnam, S.; Snowden, K.C. *Petunia hybrida* CAROTENOID CLEAVAGE DIOXYGENASE7 is involved in the production of negative and positive branching signals in petunia. *Plant Physiol.* **2009**, *151*, 1867–1877. [CrossRef] [PubMed]
24. Drummond, R.S.; Sheehan, H.; Simons, J.L.; Martínez-Sánchez, N.M.; Turner, R.M.; Putterill, J.; Snowden, K.C. The expression of petunia strigolactone pathway genes is altered as part of the endogenous developmental program. *Front. Plant Sci.* **2011**, *2*, 115. [CrossRef]

25. Hamiaux, C.; Drummond, R.S.; Janssen, B.J.; Ledger, S.E.; Cooney, J.M.; Newcomb, R.D.; Snowden, K.C. DAD2 is an α/β hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone. *Curr. Biol.* **2012**, *22*, 2032–2036. [CrossRef]
26. Beveridge, C.A.; Kyozuka, J. New genes in the strigolactone-related shoot branching pathway. *Curr. Opin. Plant Biol.* **2010**, *13*, 34–39. [CrossRef]
27. Wang, Y.; Li, J. Branching in rice. *Curr. Opin. Plant Biol.* **2011**, *14*, 94–99. [CrossRef]
28. Al-Babili, S.; Bouwmeester, H.J. Strigolactones, a novel carotenoid-derived plant hormone. *Annu. Rev. Plant Biol.* **2015**, *66*, 161–186. [CrossRef]
29. Waters, M.T.; Gutjahr, C.; Bennett, T.; Nelson, D.C. Strigolactone signaling and evolution. *Annu. Rev. Plant Biol.* **2017**, *68*, 291–322. [CrossRef]
30. Gomez-Roldan, V.; Fermas, S.; Brewer, P.B.; Puech-Pagès, V.; Dun, E.A.; Pillot, J.P.; Letisse, F.; Matusova, R.; Danoun, S.; Portais, J.C.; et al. Strigolactone inhibition of shoot branching. *Nature* **2008**, *455*, 189–194. [CrossRef]
31. Umehara, M.; Hanada, A.; Yoshida, S.; Akiyama, K.; Arite, T.; Takeda-Kamiya, N.; Magome, H.; Kamiya, Y.; Shirasu, K.; Yoneyama, K.; et al. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **2008**, *455*, 195–200. [CrossRef] [PubMed]
32. Matusova, R.; Rani, K.; Verstappen, F.W.; Franssen, M.C.; Beale, M.H.; Bouwmeester, H.J. The strigolactone germination stimulants of the plant-parasitic Striga and Orobanche spp. are derived from the carotenoid pathway. *Plant Physiol.* **2005**, *139*, 920–934. [CrossRef] [PubMed]
33. Alder, A.; Jamil, M.; Marzorati, M.; Bruno, M.; Vermathen, M.; Bigler, P.; Ghisla, S.; Bouwmeester, H.; Beyer, P.; Al-Babili, S. The path from β-carotene to carlactone, a strigolactone-like plant hormone. *Science* **2012**, *335*, 1348–1351. [CrossRef] [PubMed]
34. Wang, Y.; Shang, L.; Yu, H.; Zeng, L.; Hu, J.; Ni, S.; Rao, Y.; Li, S.; Chu, J.; Meng, X.; et al. A strigolactone biosynthesis gene contributed to the green revolution in rice. *Mol. Plant* **2020**, *13*, 923–932. [CrossRef] [PubMed]
35. Bhoi, A.; Yadu, B.; Chandra, J.; Keshavkant, S. Contribution of strigolactone in plant physiology, hormonal interaction and abiotic stresses. *Planta* **2021**, *254*, 28. [CrossRef]
36. Abe, S.; Sado, A.; Tanaka, K.; Kisugi, T.; Asami, K.; Ota, S.; Kim, H.I.; Yoneyama, K.; Xie, X.; Ohnishi, T.; et al. Carlactone is converted to carlactonoic acid by MAX1 in Arabidopsis and its methyl ester can directly interact with AtD14 in vitro. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 18084–18089. [CrossRef]
37. Yao, R.; Ming, Z.; Yan, L.; Li, S.; Wang, F.; Ma, S.; Yu, C.; Yang, M.; Chen, L.; Chen, L.; et al. DWARF14 is a non-canonical hormone receptor for strigolactone. *Nature* **2016**, *536*, 469–473. [CrossRef]
38. Goulet, C.; Klee, H.J. Climbing the branches of the strigolactones pathway one discovery at a time. *Plant Physiol.* **2010**, *154*, 493–496. [CrossRef]
39. Johnson, X.; Brcich, T.; Dun, E.A.; Goussot, M.; Haurogné, K.; Beveridge, C.A.; Rameau, C. Branching genes are conserved across species. Genes controlling a novel signal in pea are coregulated by other long-distance signals. *Plant Physiol.* **2006**, *142*, 1014–1026. [CrossRef]
40. Zou, J.; Zhang, S.; Zhang, W.; Li, G.; Chen, Z.; Zhai, W.; Zhao, X.; Pan, X.; Xie, Q.; Zhu, L. The rice HIGH-TILLERING DWARF1 encoding an ortholog of Arabidopsis MAX3 is required for negative regulation of the outgrowth of axillary buds. *Plant J.* **2006**, *48*, 687–698. [CrossRef]
41. Vogel, J.T.; Walter, M.H.; Giavalisco, P.; Lytovchenko, A.; Kohlen, W.; Charnikhova, T.; Simkin, A.J.; Goulet, C.; Strack, D.; Bouwmeester, H.J.; et al. SlCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. *Plant J.* **2010**, *61*, 300–311. [CrossRef] [PubMed]
42. Bruno, M.; Hofmann, M.; Vermathen, M.; Alde, A.; Beyer, P.; Al-Babili, S. On the substrate- and stereospecificity of the plant carotenoid cleavage dioxygenase 7. *FEBS Lett.* **2014**, *588*, 1802–1807. [CrossRef] [PubMed]
43. Pan, X.; Zheng, H.; Zhao, J.; Xu, Y.; Li, X. ZmCCD7/ZpCCD7 encodes a carotenoid cleavage dioxygenase mediating shoot branching. *Planta* **2016**, *243*, 1407–1418. [CrossRef] [PubMed]
44. Sun, Q. Research on the Function of Strigolactones in the Regulation of Shoot Branching in Tomato. Master's Thesis, Zhejiang University, Hangzhou, China, 2020.
45. Galili, S.; Hershenhorn, J.; Smirnov, E.; Yoneyama, K.; Xie, X.; Amir-Segev, O.; Bellalou, A.; Dor, E. Characterization of a chickpea mutant resistant to *Phelipanche aegyptiaca* Pers. and *Orobanche crenata* Forsk. *Plants* **2021**, *10*, 2552. [CrossRef] [PubMed]
46. Jiang, Q.; Hou, J.; Hao, C.; Wang, L.; Ge, H.; Dong, Y.; Zhang, X. The wheat (*T. aestivum*) sucrose synthase 2 gene (TaSus2) active in endosperm development is associated with yield traits. *Funct. Integr. Genom.* **2011**, *11*, 49–61. [CrossRef]
47. Aldrich, C. CTAB DNA extraction from plant tissues. *Plant Mol. Biol. Rep.* **1993**, *11*, 128–141. [CrossRef]
48. Sang, D.; Chen, D.; Liu, G.; Liang, Y.; Huang, L.; Meng, X.; Chu, J.; Sun, X.; Dong, G.; Yuan, Y.; et al. Strigolactones regulate rice tiller angle by attenuating shoot gravitropism through inhibiting auxin biosynthesis. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 11199–11204. [CrossRef]
49. Appiano, M.; Catalano, D.; Martínez, M.S.; Lotti, C.; Zheng, Z.; Visser, R.G.; Ricciardi, L.; Bai, Y.; Pavan, S. Monocot and dicot MLO powdery mildew susceptibility factors are functionally conserved in spite of the evolution of class-specific molecular features. *BMC Plant Biol.* **2015**, *15*, 257. [CrossRef]
50. Qin, X.; Fischer, K.; Yu, S.; Dubcovsky, J.; Tian, L. Distinct expression and function of carotenoid metabolic genes and homoeologs in developing wheat grains. *BMC Plant Biol.* **2016**, *16*, 155. [CrossRef]

51. Colasuonno, P.; Lozito, M.L.; Marcotuli, I.; Nigro, D.; Giancaspro, A.; Mangini, G.; De Vita, P.; Mastrangelo, A.M.; Pecchioni, N.; Houston, K.; et al. The carotenoid biosynthetic and catabolic genes in wheat and their association with yellow pigments. *BMC Genom.* **2017**, *18*, 122. [CrossRef]
52. Maeda, M.; Uryu, N.; Murayama, N.; Ishii, H.; Ota, M.; Tsuji, K.; Inoko, H. A simple and rapid method for HLA-DP genotyping by digestion of PCR-amplified DNA with allele-specific restriction endonucleases. *Hum. Immunol.* **1990**, *27*, 111–121. [CrossRef]
53. Bungartz, A.; Klaus, M.; Mathew, B.; Léon, J.; Naz, A.A. Development of new SNP derived cleaved amplified polymorphic sequence marker set and its successful utilization in the genetic analysis of seed color variation in barley. *Genomics* **2016**, *107*, 100–107. [CrossRef] [PubMed]
54. Matuszczak, M.; Spasibionek, S.; Gacek, K.; Bartkowiak-Broda, I. Cleaved amplified polymorphic sequences (CAPS) marker for identification of two mutant alleles of the rapeseed BnaA.FAD2 gene. *Mol. Biol. Rep.* **2020**, *47*, 7607–7621. [CrossRef] [PubMed]
55. Chen, S.; Hegarty, J.; Shen, T.; Hua, L.; Li, H.; Luo, J.; Li, H.; Bai, S.; Zhang, C.; Dubcovsky, J. Stripe rust resistance gene Yr34 (synonym Yr48) is located within a distal translocation of *Triticum monococcum* chromosome $5A^{m}L$ into common wheat. *Theor. Appl. Genet.* **2021**, *134*, 2197–2211. [CrossRef]
56. Sasaki, A.; Ashikari, M.; Ueguchi-Tanaka, M.; Itoh, H.; Nishimura, A.; Swapan, D.; Ishiyama, K.; Saito, T.; Kobayashi, M.; Khush, G.S.; et al. Green revolution, a mutant gibberellin-synthesis gene in rice. *Nature* **2002**, *416*, 701–702. [CrossRef]
57. Peng, J.; Richards, D.E.; Hartley, N.M.; Murphy, G.P.; Devos, K.M.; Flintham, J.E.; Beales, J.; Fish, L.J.; Worland, A.J.; Pelica, F.; et al. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **1999**, *400*, 256–261. [CrossRef]
58. Xie, Q.; Mayes, S.; Sparkes, D.L. Optimizing tiller production and survival for grain yield improvement in a bread wheat × spelt mapping population. *Ann. Bot.* **2016**, *117*, 51–66. [CrossRef]
59. Sakamoto, T.; Matsuoka, M. Identifying and exploiting grain yield genes in rice. *Curr. Opin. Plant Biol.* **2008**, *11*, 209–214. [CrossRef]
60. Donald, C.M. The breeding of crop ideotypes. *Euphytica* **1968**, *17*, 385–403. [CrossRef]