CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Analysis of allelic variation of *TaNCED1-5B* and functional marker development for drought resistance in wheat

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Abstract: *NCED1 (9-cis-epoxy carotenoid dioxygenase) plays important roles in controlling ABA levels and drought stress tolerance. The relationships between sequence polymorphisms of the TaNCED1 gene and drought resistance in wheat were analysed. Four allelic variations of the TaNCED1-5B generated to drought resistance were identified. Four groups of KASP (kompetitive allele-specific PCR) markers were developed based on the four alleles and were verified in a natural population of 311 wheat cultivars. The distribution of the four alleles of TaNCED1-5B in the natural population was clarified. Among the allelic variations, 84.62% of the wheat varieties with TT/-/C/-, TT/C/C/-, TT/-/C/G or TT/C/C/G haplotypes were dryland cultivars. The common feature of the above four haplotypes is that they all contain the TT (98) and C (343) alleles at the TaNCED1-5B-TT/CG (98) and TaNCED1-5B-C/T (343) loci, which indicated that these two locis had highly positive correlation with drought resistance in wheat.*

Keywords: *Allelic variation, drought resistance, KASP, TaNCED1, wheat*

INTRODUCTION

The shortage of water resources has become a key factor that limits the yield potential of wheat (*Triticum aestivum* L.). With the development of new genomics methods, mining and utilizing beneficial drought resistance gene resources has become one of the most important methods in breeding drought-resistant wheat cultivars (Ju et al. 2013, Zhang et al. 2014a). However, drought resistance is a complex quantitative trait which is difficult to make a breakthrough in drought resistance using traditional breeding methods. Molecular marker-assisted selection can potentially address these limitations. Single nucleotide polymorphisms (SNPs) have the advantages of large numbers, wide distribution, strong stability, and easy typing (Kassa et al. 2014). More recently, the kompetitive allele-specific PCR (KASP) system has been widely used in high-throughput SNP typing and indel detection given its advantages of high stability, accuracy, high efficiency, and cost-effectiveness (Singh et al. 2019, Grewal et al. 2020, Wang et al. 2020).

ABA is an important signaling molecule and plays an important role in drought resistance in higher plants. NCED (9-cis-epoxy carotenoid dioxygenase) is the key rate-limiting enzyme in ABA biosynthesis pathway (Qin and Zeevaart 2002, Nambara and Marion-Poll 2005). The *TaNCED1* gene was cloned from wheat,

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which contains an open reading frame of 1,848 bp and encodes a peptide of 615 amino acids (Zhang et al. 2014b). It is differentially expressed in various organs and is up-regulated in response to low temperature, drought, NaCl, and ABA. The drought tolerance of transgenic tobacco was significantly improved by over expression of *TaNCED1* (Zhang et al. 2014b). The *TaNCED1* expression levels in different wheat cultivars were found to differ significantly under drought stress, and the effects of *TaNCED1* on ABA also differed among the cultivars (Song et al. 2019). In this study, the relationship between *TaNCED1* sequence polymorphism and drought resistance in different wheat cultivars were analysed. The KASP markers were developed and could be useful in wheat drought tolerance breeding.

MATERIAL AND METHODS

Plant materials

The wild diploid wheat species *Triticumurartu* (A genome donor), *Aegilopsspeltoides* (B genome donor) and *Ae.tauschii* (D genome donor) (provided by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences) were used for the chromosome localization.

Sixteen wheat cultivars belonging to two groups were used to exemine the sequence polymorphisms of the *TaNCED1* gene. One group comprised of 10 irrigated cultivars, including 'Jimai 21', 'Weimai 8', 'Zhengmai 366', and etc. The other group included 6 drought-resistant cultivars, including 'Heshangtou', 'Linhan 2', 'Lumai21', and etc (Song et al. 2019).

The KASP markers were verified in 311 diverse wheat cultivars, of which 189 cultivars were provided by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences and the other 122 cultivars were collected from the Huang-Huai-Hai winter wheat growing region. The wheat cultivars were divided into irrigated cultivars and dryland cultivars according to the drought resistance data generated during trials for the approval of wheat varieties. Drought-resistant, drought-tolerant, water-saving, water logging-tolerant and salt-tolerant cultivars are considered to be water stress-tolerant cultivars. The natural population of 311 wheat cultivars was classified based on the above principles into 62 dryland cultivars and 249 irrigated cultivars.

DNA extraction

Genomic DNA was extracted from the leaves of wheat seedlings using the DNAquick Plant System (non-centrifugal column) (Tiangen, Beijing).

PCR amplification of the*TaNCED1* gene

PCR amplifications were performed with the NCED1-P1-F/R primer pair and *TransStart*^{O} *FastPfu* DNA Polymerase (*TransGene*, Beijing). The PCR amplification was performed as described (Zhang et al. 2014b).

Cloning and sequencing of the *TaNCED1* gene

DNA fragments of ~1,800 bp were recovered from the gel and cleaned using the UNIQ-10 column DNA gel Extraction Kit (Sangon Biotech, Shanghai). The purified 1.8-kb fragments were sub-cloned using the pEASY-Blunt Cloning Kit (TransGen Biotech, Beijing) and transformed into *E. coli* DH5α competent cells. Positive clones were detected via PCR amplification using M13F/M13R primers, and the DNA fragments were sent to Sangon Biotech and BGI for sequencing with M13F, M13R, and T7 promoter primers.

Sequence analysis of the *TaNCED1* gene

The obtained *TaNCED1* sequences were analyzed with the help of the EnsemblPlants (http://plants.ensembl.org/Triticum_aestivum/Info/Index) and the International Wheat Genome Sequencing Consortium (IWGSC) database. The sequences could be distinguished and divided into three groups according to the *TaNCED1* sequence differences of the A, B, and D genome. The sequence alignment was performed by the DNAMan software.

Design and sequences of KASP primers

Approximately 60 bp up (5') and down (3') stream of the SNP locations of the *TaNCED1-5B* gene was selected and compared to the corresponding sequences of the A and D genome. Three primers for each SNP/indel site including two

allele-specific forward primers and one common reverse primer were designed. FAM (6-carboxy-fluorescein) or HEX (hexachloro-fluorescein) fluorescent labels were added to the 5'ends of the two KASP forward primer sequences (Table 1).

KASP assays

KASP assays were performed in 1-µL reactions containing 50-100 ng genomic DNA, 0.5 µL 2×KASP Master mix, 0.014 µL 72×Assay mix, and 0.486 µL ddH_2O. ddH_2O was used as the blank control (NTC). PCR was performed as follows: 94 °C for 15 min, 10 step-down cycles of 94 °C for 20s and 61-55 °C for 60 s (decreasing by 0.6 °C each cycle), followed by 26 cycles of 94 °C for 20 s and 55 °C for 60s. A PHERAstar microplate reader (BMG Labtech) was used for KASP marker fluorescence detection. The SNPviewer software was used for KASP genotyping. The cultivars coloured blue have the FAM-type allele; cultivars coloured red have the HEX-type allele; pink dots represent undetermined; black dots represent the non-template control (NTC).

Table 1. Names and nucleotide sequences of KASP primers used for detecting sequence variants in the wheat *TaNCED1-5B* gene

Primer	Primer sequence (5'-3')	Locus	Location
TaNCED1-1-B-FAM	CGCCAGCTCGGTCCGGTTT		
TaNCED1-1-B-HEX	GCCAGCTCGGTCCGGTTC	TT/CG	At 98 bp
TaNCED1-1-B-Common	GTGCTGGTGGAGGCGGCGAT		
TaNCED1-2-B-FAM	GCGCGCTGGAAGAAGTTGAG		
TaNCED1-2-B-HEX	CGCGCGCTGGAAGAAGTTGAA	C/T	At 285 bp
TaNCED1-2-B-Common	AAGCAGAGCGGCAGCGGCAA		
TaNCED1-3-B-FAM	GCCGCTCCAGGACATTGTGGA		
TaNCED1-3-B-HEX	CCGCTCCAGGACATTGTGGG	C/T	At 343 bp
TaNCED1-3-B-Common	TTCCAGCGCGCGGCGGCG		
TaNCED1-4-B-FAM	TCGCCCCCGTCGGCGAT		
TaNCED1-4-B-HEX	CGCCCCCGTCGGCGAG	G/T	At 431 bp
TaNCED1-4-B-Common	CGTGCGTAGACGCCGTTGATGAA		

RESULTS AND DISCUSSION

Chromosomal locations of the*TaNCED1* genes

The *TaNCED1* genes isolated from the *T. urartu* (A genome donor), *Ae. speltoides* (B genome donor), and *Ae. tauschii* (D genome donor) were 1,845 bp, 1,845 bp and 1,848 bp in length and shared 99%, 96% and 96% sequence homology, respectively. The three sequences of *TaNCED1-1*, *TaNCED1-2*, and *TaNCED1-3* which were cloned from the hexaploid 'Jimai 21'could be aligned to the diploid wild relatives of wheat *T.urartu* (AA, 2n = 2x = 14), *Ae.speltoides* (BB, 2n = 2x = 14), and *Ae. Tauschii* (DD, 2n = 2x = 14), respectively. Accordingly, we named the corresponding sequences as *TaNCED1-A, TaNCED1-B*, and *TaNCED1-D*.

The EnsemblPlants (http://plants.ensembl.org/Triticum_aestivum/Info/Index) and International Wheat Genome Sequencing Consortium (IWGSC) databases were used to analyse the obtained *TaNCED1* sequences. The *TaNCED1* genes consisted of a single uninterrupted exon. The sequences of *TaNCED1-A, TaNCED1-B*, and *TaNCED1-D* were good matches to three surveyed sequences on chromosomes 5AS (99%), 5BS (99%), and 5DS (100%) of 'Chinese Spring' (Figure 1), and they are named as *TaNCED1-5A, TaNCED1-5B* and *TaNCED1-5D,* respectively.

The *TaNCED1* sequences from 16 wheat cultivars that differ in drought resistance were aligned with the sequences of 5AS, 5BS and 5DS. The *TaNCED1* sequences of the A, B and D genome of each wheat cultivar were mapped. The sequences of *TaNCED1-5A*, *TaNCED1-5B*, and *TaNCED1-5D* from the 16 wheat cultivars were then obtained.

Analysis of allelic variations in the*TaNCED1-5B* gene

The sequences of the *TaNCED1-5A*, *TaNCED1-5B*, and *TaNCED1-5D* genes from wheat cultivars with different drought resistance were subjected to DNA sequence alignment. Sequence polymorphisms present in the *TaNCED1-*

5A, *TaNCED1-5B*, and *TaNCED1-5D* genes were noted in the different drought-resistant cultivars. Further analysis revealed that allelic variations were present in the *TaNCED1-5B* gene between the irrigated cultivars and the dry land cultivars. However, the complex nature of the variations made it difficult to determine whether they belonged to dry land cultivars or irrigated cultivars. To further clarify this issue, we selected 'Heshangtou', a typical drought-tolerant cultivar, as the reference standard. The *TaNCED1-5B* sequence from 'Heshangtou' was used as a reference sequence in the analysis of the sequence polymorphisms in the *TaNCED1-5B* gene between the dry land and irrigated cultivars. And four loci were found to be associated with drought resistance. These alleles were located at positions 98 bp, 285 bp, 343 bp and 431 bp in the *TaNCED1-5B* gene, and were TT/CG, C/T, C/T, and G/T, respectively (Figure 2). The alleles of the above four loci were named *TaNCED1-5B-TT* (98), *TaNCED1-5B-CG* (98), *TaNCED1-5B-C* (285), *TaNCED1-5B-T* (285), *TaNCED1-5B-C* (343), *TaNCED1-5B-T* (343), *TaNCED1-5B-G* (431), and *TaNCED1-5B-T* (431). The *TaNCED1-5B-*

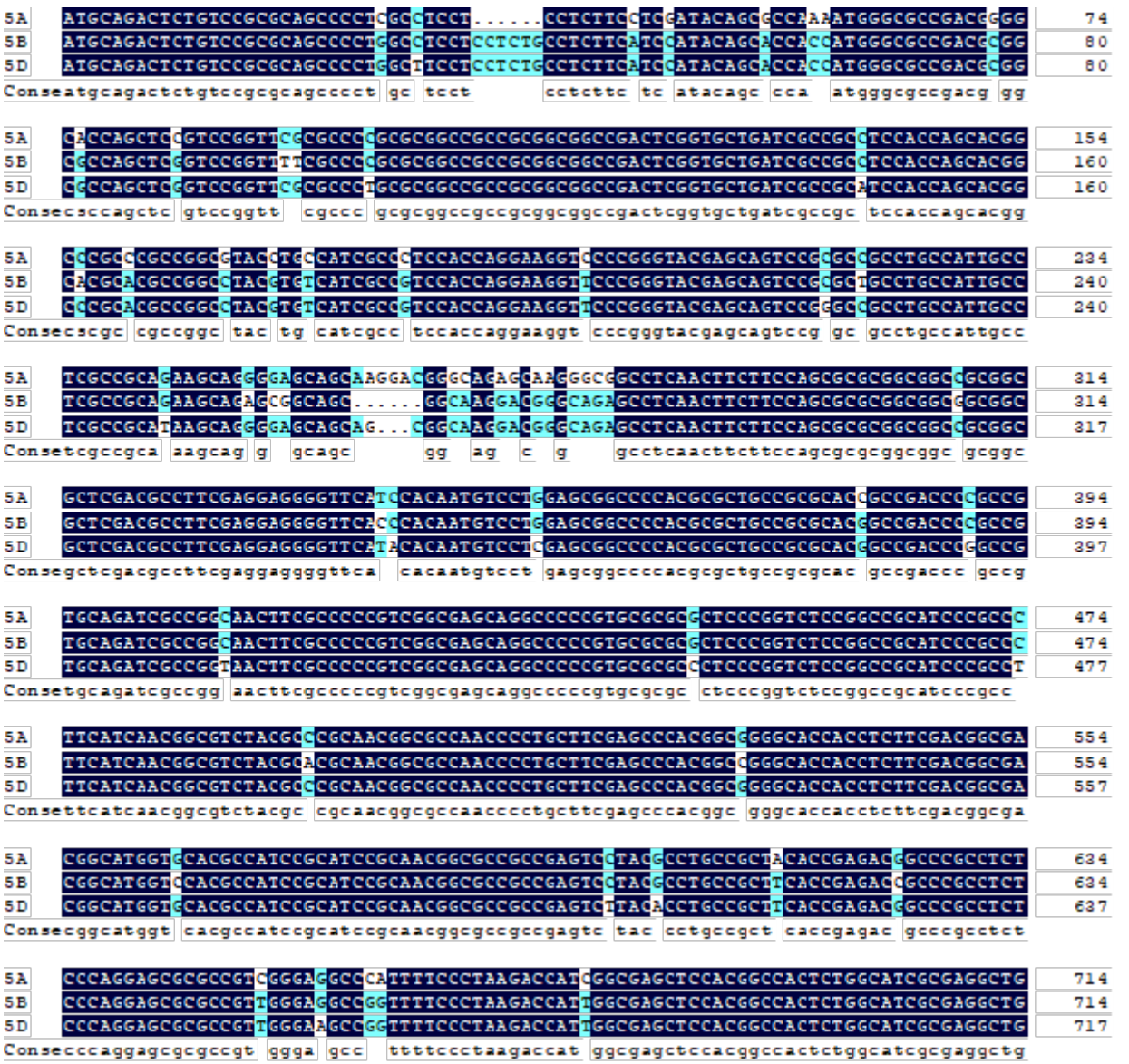

Figure 1. Sequence differences in the *TaNCED1*genes from the A, B, and D genomes of wheat. Fully conserved nucleotides are highlighted in dark blue, and polymorphisms common to two genes are highlighted in light blue.

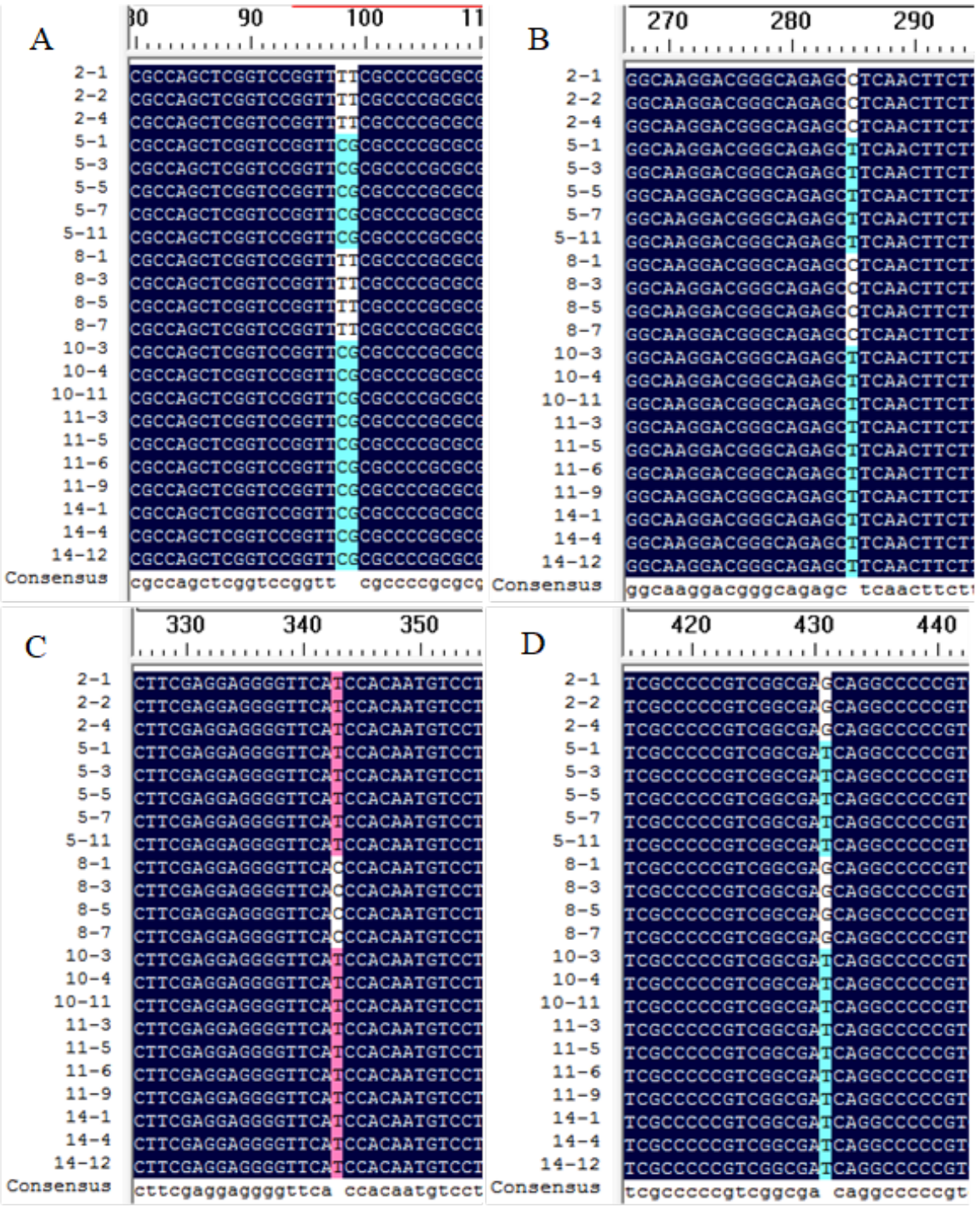

Figure 2. Allelic sequence variations in the *TaNCED1-5B* gene. The wheat cultivars in the four panels are identified by the following codes: 2, 'Linhan 2'; 5, 'Qingmai 6'; 8, 'Heshangtou'; 10, 'Zhengmai 366'; 11, 'Lumai 21'; 14, 'Weimai 8'. The number after the dash indicates the number of the individual cloned sequence. Panels A-D show the nucleotide polymorphisms in four 30-bp regions of the *TaNCED1-5B* gene.

TT (98), *TaNCED1-5B-C* (285), *TaNCED1-5B-C* (343), and *TaNCED1-5B-G* (431) from 'Heshangtou' represent beneficial to drought resistance.

Distribution of the *TaNCED1-5B* gene alleles

All 311 wheat cultivars were assayed using the four KASP markers (Table 1), and the genotyping map of each KASP marker was obtained using the SNPviewer software (Figure 3). The distribution of each allele in the wheat cultivars were analysed. The frequencies of the alleles *TaNCED1-5B-C* (343) and *TaNCED1-5B-T* (343) at the C/T (343) locus were 5.79% and 94.21%, respectively (Figure 3C). The distribution frequency of these two alleles in this locus has the largest differences among the four loci in surveyed wheat cultivars. On the TT/CG (98), C/T (285), and G/T (431) loci (Figures 3A, B, and D), the distribution frequencies of *TaNCED1-5B-TT* (98), *TaNCED1-5B-CG* (98), *TaNCED1-5B-C* (285), *TaNCED1-5B-T* (285), *TaNCED1-5B-G* (431), and *TaNCED1-5B-T* (431) in the wheat cultivars were 21.54%, 78.46%, 30.55%, 69.45%, 30.55%, and 69.45%, respectively.

Haplotype distribution of the *TaNCED1-5B* gene

A total of six haplotypes were identified in *TaNCED1-5B* (Table 2); these were TT/C/C/G, TT/C/T/G, TT/T/T/T, CG/C/C/G, CG/C/T/G, and CG/T/T/T. The CG/T/T/T was the most frequent polymorphic halotype among the six haplotypes, which was present in 215 wheat cultivars accounting for 69.13% of the total. The TT/T/T/T haplotype was the least frequent polymorphic one which was found only in a single cultivar (Table 2).

Relationships between allelic variation in the *TaNCED1-5B* gene and drought resistance

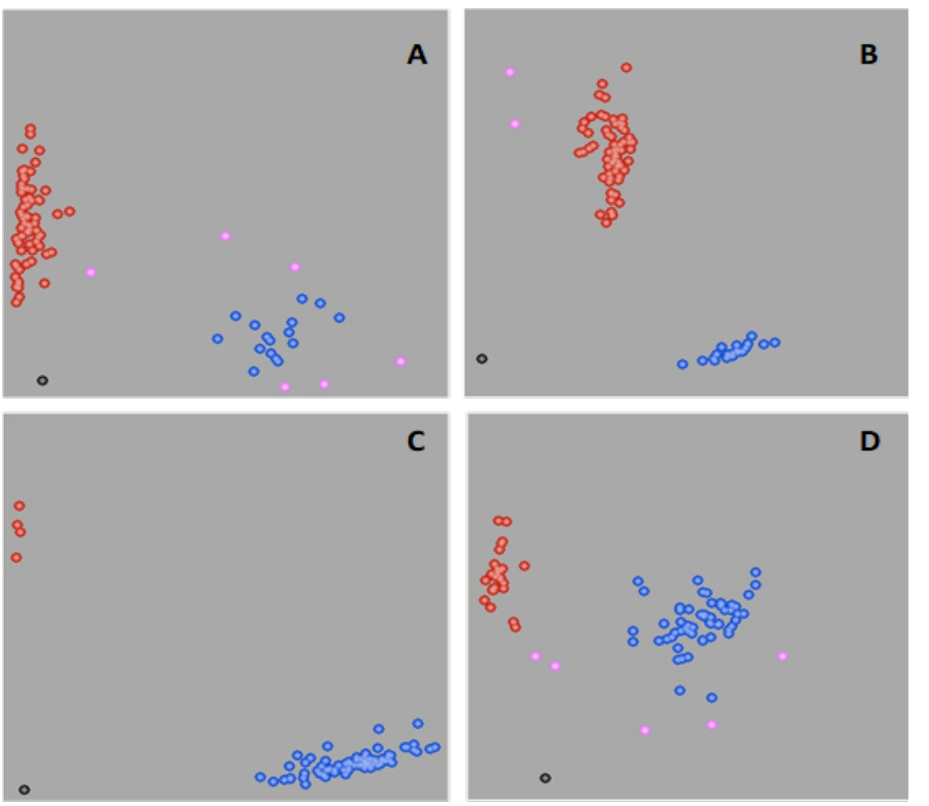

Figure 3. Scatter plots for four KASP assays showing clustering of cultivars on the X- (FAM) and Y- (HEX) axes. A. KASP assay for TaNCED1-1-B (TT/CG 98) showing TT on FAM and CG on HEX clusters; B. KASP assay for TaNCED1-2-B (C/T 285) showing C on FAM and T on HEX clusters; C. KASP assay for TaNCED1-3-B (C/T 343) showing T on FAM and C on HEX clusters; D. KASP assay for TaNCED1-4-B (T/G 431) showing T on FAM and G on HEX clusters.

Table 2. Wheat cultivars corresponding to the six different *TaNCED1-5B* gene haplotype groups

Haplotype	Wheat cultivars	Number
TT/C/C/G	Dekang 961, Heshangtou, Jinmai 1, Kanto 107, Lumai 11, Lumai 5, Shan 512, Zhengmai 04H43, Xiaoyan 22, Zhongmai 871, Zhongyu 9302, Zhongyu 9307, Zhoumai 13	13
TT/C/T/G	Beijing 0045, Beijing 841, BRUTA, Fr03725, Gan 05-5092, Hengguan 33, Hengguan 35, Henong 5480, HK1/, Jimai 0850262, Jimai 325, Jing 411, Jing 9428, Jingdong 8, Jingshuang 16, KNIISH 46, Lankao 2, Lankao 24, Libero, Linhan 2, Linkang 12, Linyou 145, Linyou 359, Linyuan 6521, Lovrin13, Lumai 9, Mason/Jagger, Mesofold, Minxian 169, Nidera Baguette 20, Ningdong 10, Nongda 211, Nongda 212, PH82-2, Sagittario, Salmone, SELYANKA, Shannong 670, Shi u10-4125, Shi u10-4152, Tainong 1014, Wan 23094, Wennong 6, Xiaoyan 81, Xinmai 37, Xinong 291, Xinong 88, Xinong 979, Xu 5034, Yumai 35, Zhongmai 415, Zhongyou 335, Zixuan 2	53
TT/T/T/T	Qinnong 151	1
CG/C/C/G	Hemai 20, Jishi 02-1, Shan 715, Zhoumai 16, Zhoumai 28	5
CG/C/T/G	BATJKO, CA1119, D, DONSKI-93, Fr3713, Genio, Guan 35, Jinmai 67, Klein Flecha, Neixiang 188, Norin 61, Qinnong 731, Shan 229, Shan 253, Shannong 78-59, Shi 4185, Shijiazhuang 15, Taishan 1, Thesee, Wanmai 33, Yannong 173, Zhongmai 175, Zhongyou 206, Zhoumai 11	24
CG/T/T/T	975106, 046097 (Yannong 19/935096), 11CA40, 3768 (Yannong 19/Liangxing 66), 4510(Tainong 18/Yannong 19), 85 Zhong 33, Abbondanza, Aca 601, Aca 801, Aikang 58, An 1331, Aztec, Azulon, Bainong 64, BFB10, Blu99603, BN10F(002) Jia 1-Te 1, C, C39, CA0548, , CA0816, CA0998, CA1055, CA1062, CA1090, CA1133, CA1135, CA9722, Carimulti, Changwu 134, Cunmai 1, Darius, Denghai 206, Dorico, F92080G1-1/F93042G2-1, Fengchan 3, Fr03711, Fr03724, Fr03732, Fr03733, Fu 936, Gaocheng 8901, Gaoyou 2018, Gaoyou 5218, Gaoyou 5766, Han 05-5093, Han 09-41344, Han 6172, Hemai 0643, Hemai 0746-2, Hemai 0839, Hemong 583, Heng 7228, Henong 7069, Huaimai 18, Huaimai 21, Insignia, Jagger/W94-244-132, Jimai 181, Jimai 21, Jimai 22, Jimai 23, Jimai 262, Jinan 13, Jinan 17, Jingdong 17, Jingyang 669, Jinmai 45, JM23, JN331, JNM3070, K03566, Keheng 6654, Kitanokaori, Klein Jabal 1, Lampo, Lankao 351, Lankao 381, lasen, Liangxing 518, Liangxing 66, Liangxing 99, Linmai 4, Linmai 6, Linxuan 5186, Linyuan 3158, Lovrin10, LS3639, LS4035, LS4211, LS4695, LS4942, Lumai 14, Lumai 15, Lumai 21, Lumai 23, Lumai 6, Lumai 7, Lumai 8, Lunxuan 987, Luomai 21, Magnus, Mantol, MASON/JAGGER, MV LAURA, Nidera Baguette 10, Ningdong 11, Nongda 139, Norin 67, PALPICH, ProINTAColibr 1, Puxing 5, Qidu 7, Qifeng 2, Qimai 2, Qingmai 6, Qingmai 7, Qinmai 5, Rumai 1, SH4300, SH5099, SH5186, SH5195, Shaan 150, Shaan 354, Shaanmai 509, Shaanmai 94, Shaannong 981, Shannong 0713-2, Shannong 0911, Shannong 15, Shannong 20, Shannong 22, Shannong 23, Shannong 24, Shannong 25, Shannong 29, Shanrong 3, Shengmai 102, Shi u09-4366, Shixin 733, Shixin 828, Soissons, STARSHINA, Su 0663, Su 553, Sunstate, Tai 7087, Taimai 18, Tainong 8968, Taishan 21, Taishan 22, Taishan 23, Taishan 4241, Taishan 4606, Taishan 5, Taishan 6436, Taishan 7087, Wanmai 29, Wanmai 38, Wanmai 50, Wanmai 52, Wanmai 53, Weimai 7, Wennong 14, Wennong 17, Wennong 19, Wennong 28, Wennong 5, WGRC10/3/KS93U69, Xin 18391, Xin 19323, Xingmai 13, Xinmai 19, Xinmai 26, Xinmai 296, Xinmai 9, Xinmai 9408, Xinong 2000-7, Yan 1212, Yan 85771, YANA, Yangmai 15, Yannong 18, Yannong 19, Yannong 578, Yannong 999, Yanzhan 4110, Yong 4896, Yumai 18, Yumai 2, Yumai 47, Yumai 49, Yumai 50, Yumai 57, Yumai 63, Yunfengyou 1, Yutianmai 119, YX11-57, Zheng 91138, Zhengmai 366, Zhong 892, Zhongmai 1, Zhongmai 23, Zhongmai 4072, Zhongxin 6285, Zhongxinmai 99, Zhongyou 9507, Zhongyu 5, Zhongyu 9, Zhoumai 22, Zhoumai 25, Zhoumai 31, Zhoumai 32, Zhouyuan 9369	215

The relationship between nucleotide polymorphisms of the *TaNCED1-5B* gene and drought resistance of the 311 wheat cultivars were analyzed. The results showed that the proportion of dryland cultivars carrying the *TaNCED1-5B-C* (343) allele was as high as 61.11% (Table 3). Among the allelic variations, 84.62% of the wheat varieties with TT/-/C/-, TT/C/C/-, TT/-/C/G or TT/C/C/G halotypes were dryland cultivars. The common feature of the above four haplotypes is that they all contain the TT (98) and C (343) alleles at the TaNCED1-5B-TT/CG (98) and TaNCED1-5B-C/T (343) loci, which indicated that these two locis had highly positive correlation with drought resistance in wheat. The KASP markers for the TT/CG (98) and C/T (343) loci could be used as markers for screening drought- and salt-tolerant wheat cultivars.

Common wheat (*Triticum aestivum* L.) is hexaploidy with a large and complex genome. There are compensatory effects between the A, B, and D genomes (IWGSC 2014, Choulet et al. 2014, Marcussen et al. 2014). Drought resistance in wheat is a complex trait controlled by multiple genes. In this study, we found that single-base pair variations in the *TaNCED1-5B* gene can change drought resistance in wheat, and variations in two loci can significantly improve drought resistance. However, the haplotypes of the *TaNCED1-5B* gene were not completely consistent with the drought resistance phenotype, which was consistent with previous studies such as *TaDREB1*, *TaCRT-D*, *TaPK7*, *TaFer-A1*, and *TaNRX* genes (Chen et al. 2005, Wang et al. 2008, Zhang et al. 2008, Ju et al. 2013, Zhang et al. 2014a). Drought resistance is a complex and quantitative trait. It is difficult to completely determine the drought resistance phenotype based on the nucleotide polymorphism present in a single gene (Chen et al. 2005). However, compared with the traditional methods, molecular marker genotyping is simpler and faster in the identification of drought resistant cultivars, and can provide an effective selection method for drought resistance breeding in wheat.

Table 3. Analysis of the relationships between nucleotide polymorphisms in *TaNCED1-5B* and the percentage of dryland wheat cultivars

Allelic variations combination	Allelic variations or haplotypes	Total Number of cultivars	Number of dryland cultivars	Number of irrigated cultivars	Percentage of dryland cultivars
TT/CG (98)	TT/-/-/-	67	28	39	41.79
	CG/-/-/-	244	34	210	13.93
C/T (285)	-/C/-/-	95	34	61	35.79
	-/T/-/-	216	28	188	12.96
C/T (343)	-/-/C/-	18	11	7	61.11
	-/-/T/-	293	51	242	17.41
G/T (431)	-/-/-/G	95	34	61	35.79
	-/-/-/T	216	28	188	12.96
TT/CG (98) and C/T (285)	TT/C/-/-	66	28	38	42.42
	CG/C/-/-	29	6	23	20.69
	TT/T/-/-	1	0	1	0.00
	CG/T/-/-	215	28	187	13.02
TT/CG (98) and C/T (343)	**TT/-/C/-**	**13**	**11**	**2**	**84.62**
	CG/-/C/-	5	0	5	0.00
	TT/-/T/-	54	17	37	31.48
	CG/-/T/-	239	34	205	14.23
TT/CG (98) and G/T (431)	TT/-/-/G	66	28	38	42.42
	CG/-/-/G	29	6	23	20.69
	TT/-/-/T	1	0	1	0.00
	CG/-/-/T	215	28	187	13.02
C/T (285) and C/T (343)	-/C/C/-	18	11	7	61.11
	-/C/T/-	77	23	54	29.87
	-/T/T/-	216	28	188	12.96
C/T (285) and G/T (431)	-/C/-/G	95	34	61	35.79
	-/T/-/T	216	28	188	12.96
C/T (343) and G/T (431)	-/-/C/G	18	11	7	61.11
	-/-/T/G	77	23	54	29.87
	-/-/T/T	216	28	188	12.96
TT/CG (98) and C/T (285) and C/T (343)	**TT/C/C/-**	**13**	**11**	**2**	**84.62**
	CG/C/C/-	5	0	5	0.00
	TT/C/T/-	53	17	36	32.08
	CG/C/T/-	24	6	18	25.00
	TT/T/T/-	1	0	1	0.00
	CG/T/T/-	215	28	187	13.02
TT/CG (98) and C/T (285) and G/T (431)	TT/C/-/G	66	28	38	42.42
	CG/C/-/G	29	6	23	20.69
	TT/T/-/T	1	0	1	0.00
	CG/T/-/T	215	28	187	13.02
TT/CG (98) and C/T (343) and G/T (431)	**TT/-/C/G**	**13**	**11**	**2**	**84.62**
	CG/-/C/G	5	0	5	0.00
	TT/-/T/G	53	17	36	32.08
	CG/-/T/G	24	6	18	25.00
	TT/-/T/T	1	0	1	0.00
	CG/-/T/T	215	28	187	13.02
C/T (285) and C/T (343) and G/T (431)	-/C/C/G	18	11	7	61.11
	-/C/T/G	77	23	54	29.87
	-/T/T/T	216	28	188	12.96
TT/CG (98) and C/T (285) and C/T (343) and G/T (431)	**TT/C/C/G**	**13**	**11**	**2**	**84.62**
	TT/C/T/G	53	17	36	32.08
	TT/T/T/T	1	0	1	0.00
	CG/C/C/G	5	0	5	0.00
	CG/C/T/G	24	6	18	25.00
	CG/T/T/T	215	28	187	13.02

CONCLUSION

The sequence polymorhpisms of the *TaNCED1-5B* gene were analysed in a large collection of wheat cultivars. Four allelic variations related to drought resistance were identified. KASP markers were developed based on the four allelic variations and were verified in a natural population composed of 311 wheat cultivars originating from different regions. Among the allelic variations, 84.62% of the wheat varieties with TT/-/C/-, TT/C/C/-, TT/-/C/G or TT/C/C/G halotypes were dryland cultivars. And They all contain the TT (98) and C (343) alleles at the TaNCED1-5B-TT/CG (98) and TaNCED1-5B-C/T (343) loci. The KASP markers for the TT/CG (98) and C/T (343) loci could be used as markers for screening drought- and salt-tolerant wheat cultivars.

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