Indian J. Genet., 78(1): 11-18 (2018)
DOI: 10.5958/0975-6906.2018.00002.0

Development and validation of markers for spike density QTL, *Qsd.sau-7A* from Tibetan semi-wild wheat (*Triticum aestivum* ssp. *tibetanum*)

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(Received: September 2017; Revised: December 2017; Accepted: January 2018)

Abstract

Spike density is one of the few traits that determines the yield potential in wheat. Three high resolution melting (HRM) markers (0C32-715, DCE-464, and C81A-240) were developed and used to map the QTL for spike density *Qsd-sau-7A*. The markers 0C32-715 and DCE-464 were validated in two of the three validation population. DCE-464 and 0C32-715 were the most closely linked markers that could be used to introgress *QSd.sau-7A* through molecular maker assisted breeding and development of near isogenic lines.

Key words: Spike density, *QSd-sau-7A*, high resolution melting, validation

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world. Wheat grain yield is a product of grain number per spike, grain weight and spike number per unit area (Mengistu et al. 2012). Spike characteristics, including spike length, total spikelet number per spike, spike density and grains per spikelet determine grain number per spike and thus contributes to the yield potential (Zhou et al. 2017). Compared with spike length and the total spikelet number, spike density has been rarely focused. The spike density is a ratio of the number of grains per spike to the spike length.

Three domestication genes including *Q. compactum* (*C*), and *sphaerococcum* (*S*) are related to wheat spike morphology and have been identified on chromosomes 5A, 2D and 3D, respectively (Rao 1977; Faris et al. 2002; Faris et al. 2003; Johnson et al. 2008). The *Q* gene conferring a free-threshing spike which influences plant height, spike density, rachis toughness, and spike emergence time (Faris et al. 2003; Simons et al. 2006; Sormacheva et al. 2015). The *C* gene affects spike density, grain size, grain shape, and grain number per spike (Johnson et al. 2008). The *S* gene pleiotropically confers dense spikes, rigid short culms, straight flag leaves, hemispherical glumes, and small spherical grains (Rao 1977). In addition to these genes, numerous previous studies have identified other genomic intervals associated with spike density. For example, Jantasuriyarat et al. (2004) detected four QTLs for spike density on 1AS, 1BS, 4AL, and 7AL. Kosuge et al. (2012) found that the *C17648* gene for spike density was located on chromosome 5AL from tetraploid wheat. Ma et al. (2007) detected QTLs for spike density on 2D, 4A, 5A, 5B, 7D using recombinant inbred lines and immortalized F_2 population of Wangshuibai and Nanda2419. A QTL located on the 2AL chromosome of *T. turgidum* conferring spike density was also identified by Amagai et al. (2016). To our knowledge, these newly identified loci or genes for spike density have not been fine mapped and cloned.

Tibetan semi-wild wheat (*T. aestivum* ssp. *tibetanum*) is a hexaploid wheat found only in Tibet (Shao et al. 1980). It has unique and primitive characteristics such as hulled glumes and brittle rachis (Jiang et al. 2015; Luo et al. 2016) but also possesses numerous desirable traits including tolerance to nutrition deficiency and strong seed dormancy which could be introduced in common wheat (Sun et al. 1998; Jiang et al. 2014). Further, efforts have been made to tap

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the advantages of Tibetan semi-wild wheat in wheat breeding. Quite a few of novel QTLs governing agronomic traits have earlier been identified (Luo et al. 2016; Zhou et al. 2016b).

In our previous study, we have detected five QTLs for spike density located on chromosomes 2D (*QSd.sau-2D*, ~8% phenotypic variation explained, PVE), 3D (*QSd.sau-3D*, ~6% PVE), 5A (*QSd.sau-5A1* and *QSd.sau-5A2*, 12% and 22% PVE, respectively) and 7A (*QSd-sau-7A*, ~6% PVE), respectively (Luo et al. 2016). As the QTLs on 2D, 3D and 5A likely belong to alleles of *C*, *S* and *Q*, the study thus focused on *QSd-sau-7A* which was stably detected across several growth environments. The *QSd-sau-7A* was mapped on 7AL and the positive allele of this QTL was from Tibetan semi-wild wheat. Aiming at fine mapping and further cloning this QTL, we have been attempting at developing molecular markers. High resolution melting (HRM) analysis is a fast, consistent, and efficient method for SNP discovery and genotyping (Han et al. 2012; Wang et al. 2016; Zhou et al. 2016a; Liu et al. (2018). Here, we report on development and validation of HRM markers for *QSd.sau-7A*.

Materials and methods

Plant materials

An F_{13} recombinant inbred line (RIL) population (designated as QZ) consisting of 186 lines derived from a cross between Tibetan semi-wild wheat accession Q1028 and a common wheat cultivar Zhengmai 9023 (ZM9023) were used for construction of the genetic map for the newly developed markers (Fig. 1a and b). Three populations, QZ2×QZ13 (F_2 population, 520 lines; Fig. 1c), QZ2×ZM9023 (F_2 population, 500 lines; Fig. 1d), and Q1028×99E18 (RIL population, 180 F_{10} lines; Fig. 1e and f) were used to verify the reliability of the developed markers. The two lines QZ2 and QZ13 were from QZ population.

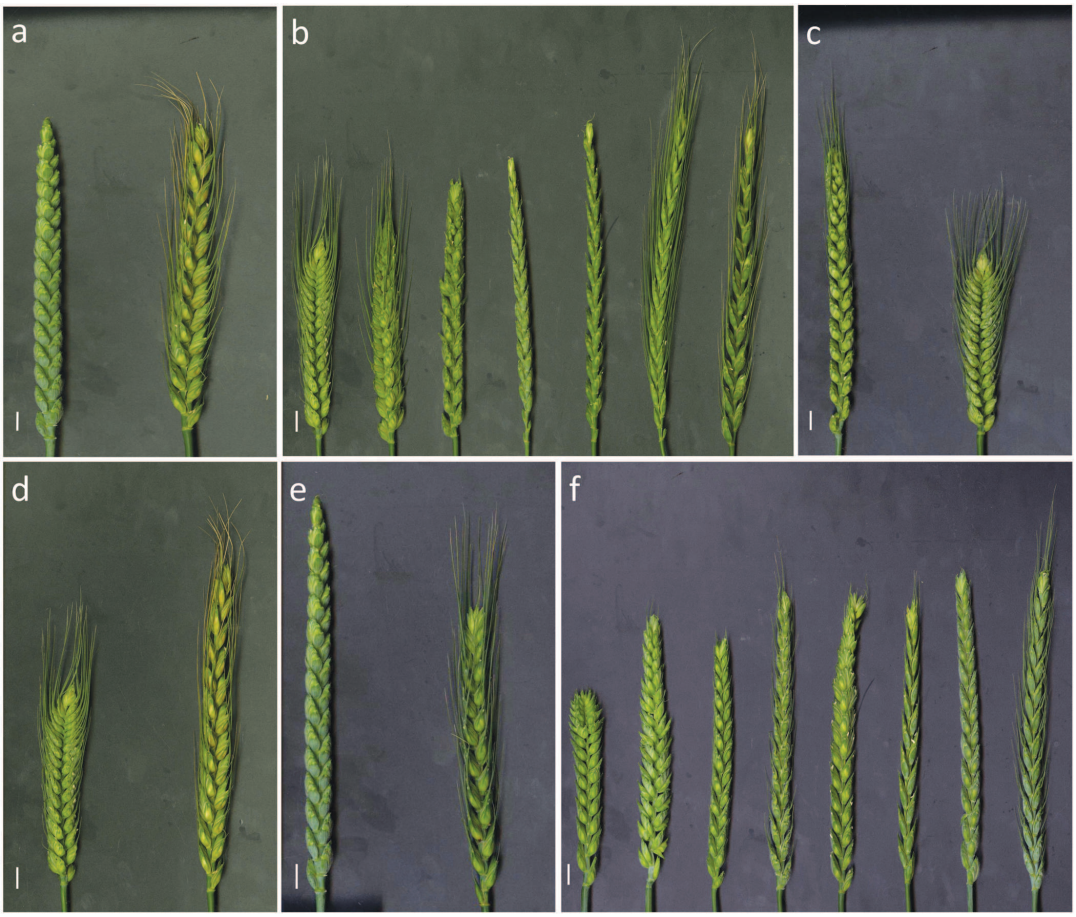

Fig. 1. Morphological features of spikes for a parents (Q1028, left; ZM9023, right) and b lines (QZ2, QZ58, QZ45, QZ46, QZ208, QZ108; left to right) of QZ RIL population; c QZ13 (left) and QZ2 (right); d QZ2 (left) and ZM9023 (right); and e parents (Q1028, left; 99E18, right) and f lines (QE184, QE243, QE212, QE225, QE172, QE175, QE249; left to right) of QE RIL population, the white scale bar represents 1 cm

The plant materials were assessed at the experimental farm of the Triticeae Research Institute, Sichuan Agricultural University, China. Each plot comprised two rows 2 m long with a spacing of 30 cm between rows and 10 cm between plants. Field management was the same as that commonly practiced in wheat production.

Phenotype measurement and data analysis

Spike density (n/cm) was measured as spikelet number per spike divided by spike length according to Luo et al. (2016). For QE population, the data were collected from two crop seasons (2009-2010 and 2010-2011). Five different plants in the middle of a row for each plot were selected for measuring spike density. The best linear unbiased prediction (BLUP) value of the data from two crop seasons was used for further analysis by SAS V8.0 (SAS Institute, Cary, North Carolina). For QZ2×QZ13 and QZ2×ZM9023 population, the data from three similar tillers for a single plant were collected in a single crop season (2015-2016) and the average of the three tillers were used for analysis.

Collection of sequence data

Shotgun genome sequences and gene models of the wheat cultivar 'Chinese Spring' were downloaded from http://wheat-urgi.versailles.inra.fr/ hosted by Unité de Recherche Génomique Info (URGI; IWGSC 2014). Assembled chromosome sequences for wheat were downloaded from Ensemblplantsv32 (ftp://ftp.ensemblgenomes.org/pub/plants/release-32/fasta/). Coding sequences (CDS) and assembled whole genome sequences of *Brachypodium distachyon* v2.0 and rice (*Oryza sativa*) were obtained from Phytozome v10 and v9.0, respectively (http://www.phytozome.org/). Whole genome shotgun sequences of T. urartu were downloaded from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) in 2013. CDS data for *T. urartu* were downloaded from GIGA_DB (http://gigadb.org/) in 2013. The contigs and gene models were downloaded from ftp://ftpmips.helmholtz-muenchen.de/plants/barley/public_data/ (International Barley Genome Sequencing Consortium 2012). The barley pseudomolecules and CDS were downloaded from http://webblast.ipk-gatersleben.de/barley_ibsc/downloads/ (Mascher et al. 2017).

Selection of genes for further isolation

The available sequences of markers including the simple sequence repeat (SSR) and Diversity arrays technology (DArT) in the constructed genetic map (Luo et al. 2016) were blasted against 'Chinese Spring (CS)' contig sequences and *T. urartu* scaffold sequences using the BLAST++ BLASTN algorithm with an E value cut-off of $0\text{-}10^{-5}$. The anchored *T. urartu* scaffolds were further retrieved to search gene sequences. The obtained genes of *T. urartu* were blasted against CDS of *B. distachyon*, rice, and barley to identify their orthologous sequences (Fig. 2). Thus, the interval collinear with the *QSd.sau-7A* region were identified in *B. distachyon*, rice, and barley. Further, the 'CS' contigs between the anchored ones (7AL_4551743 and 7AL_4465584) flanked the *QSd.sau-7A* were retrieved for searching 'CS' gene models. A total of 17 barley orthologs and 274 'CS' gene models with 9 shared gene sequences between them in the *QSd.sau-7A* region were identified. These 9 common genes were isolated for further marker development (Fig. 2). The marker sequences were also anchored on the public 'CS' assembled chromosome sequence. There were 1, 007 genes between the anchored flanking markers (i.e. wPt-5949 and wPt-0961) of *QSd.sau-7A*, 532 of which belong to single copy genes (Fig. 3). These 532 single copy genes were selected for further isolation. Thus, a total of 541 (532+9) candidate genes were further isolated.

Gene isolation and sequencing

To detect any possible polymorphism between Q1028 and ZM9023 for marker development, the above identified genes were further analyzed and isolated. Note that for detecting polymorphism conveniently and quickly, only part region of a given gene where chromosome-specific primers could be easily developed was isolated. Genomic DNA were extracted by cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). Chromosome-specific primers were designed based on our previous study (Supplementary Table S1). The PCR reaction was performed as follows: Pre-denaturation at 94°C for 4 min, then 40 cycles of 94°C for 1 min, annealing at 60~65°C for 40 s, extension at 72°C for 50 s, and a final extension at 72 °C for 7 min (Ma et al. 2015). Amplification reactions were performed in a total volume of 20μl containing 100 ng of template DNA, 10μl of 2x Taq Master Mix, and 5 pmol of each forward and reverse primer, and 8μL of ddH_2O. 'CS' nullisomic-tetrasomic lines were used to identify chromosome-specific amplification (Fig. 4, a). All of the PCR products were purified using QIAquick Extraction Kit (QIAGEN, Hilden, Germany). Then the purified fragment was cloned into PMd19-T plasmid vector.

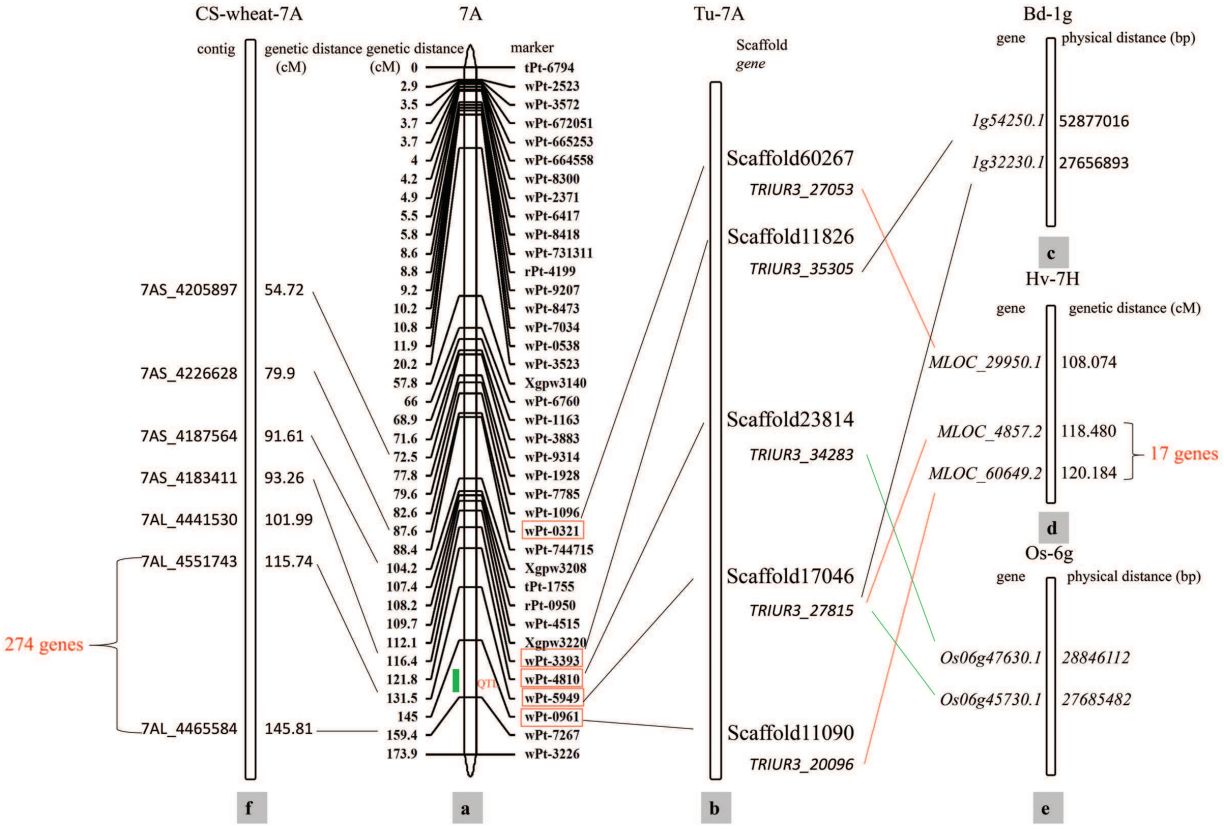

Fig. 2. Genetic maps for spike density QTL QSd.sau-7A (a)and its comparison with scaffolds of *T. urartu* (b), genomic region of *Brachypodium* (c) and rice (e), genetic maps of barley (d) and public genetic locations of 'CS' wheat contigs (f)

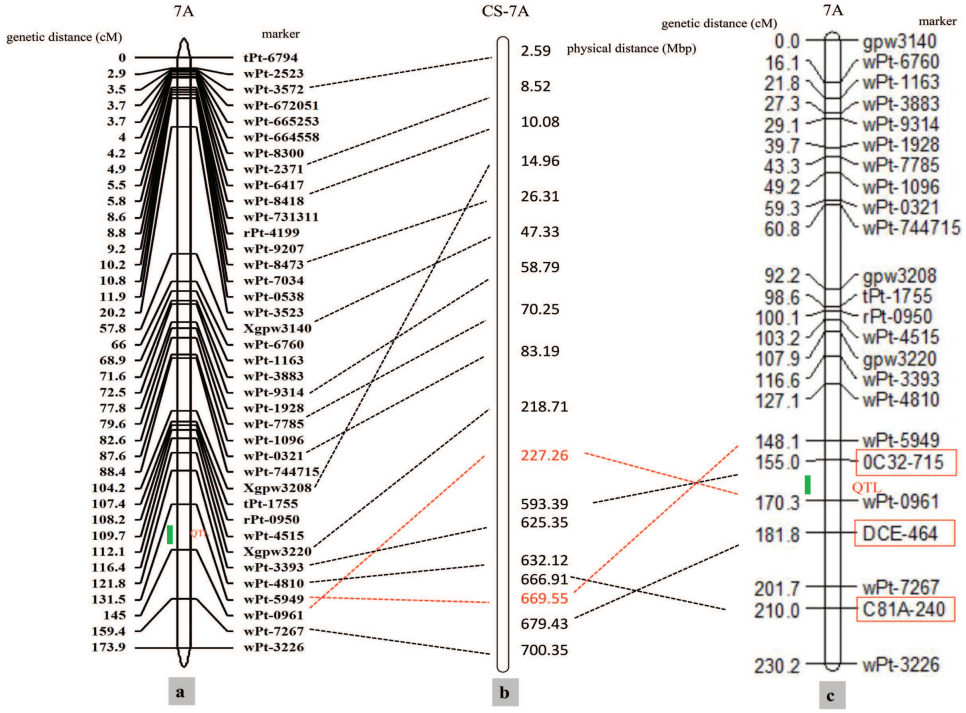

Fig. 3. Newly developed HRM markers and their genetic and physical locations; a=genetic map for spike density QTL *QSd.sau-7A*; b=physical locations for markers and c=genetic map integrated with newly developed HRM markers for *QSd.sau-7A*

The cloned fragments were then sequenced by Invitrogen Biotechnology Co. Ltd., P.R. China using at least three different independent clones in both directions.

Sequence alignment, marker development, and map construction

The obtained sequences were aligned using DNAman 7.0 (LynnonBiosoft, San Ramon, CA, USA) to detect single nucleotide polymorphisms (SNPs). These SNPs were further converted into HRM markers and single-base differences could be detected by HRM analysis (Wittwer et al. 2003; Wang et al. 2016; Zhou et al. 2016a). HRM markers were designed using Beacon Designer 7.9 and evaluated by Oligo 6.0 (Zhang and Gao 2004) (Fig. 4b; Supplementary Table S2). The parameters were set as follows: inner product size of 60-100 bp, melting temperature of 55 ± 5°C, primer length of 20 ±3 bp, and 3¹- end stability to avoid self-complementarity and primer dimer formation.

The parental DNA was firstly used to detect the efficiency of the developed markers (Fig. 4c). The

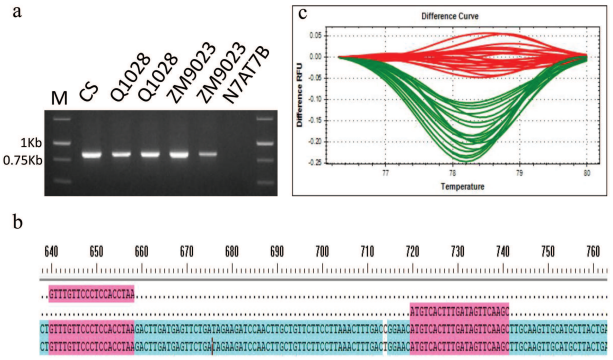

Fig. 4. Chromosome specific amplification and marker development; gene isolation (a), HRM marker development (b) and HRM analysis (c)

verified makers were further used to genotype QE, QZ, QZ2×QZ13, and QZ2×ZM9023 populations. Amplification reactions were performed in a total volume of 10μl containing 100 ng of template DNA, 5ìl of Fast Super EvaGreen mixture, and 5 pmol of each forward and reverse primer, and DNase/RNase-free water up to the final value. The PCR program includes the following steps: 4 min at 94°C, 50 cycles of 15 s at 94°C, and 30 s at 55°C. This process is a precise warming of the amplicon DNA from around 65°C up to around 95°C (Liu et al. 2018). When the temperature is reached, the DNA double strand will be melting. Based on the HRM analysis in the parental lines and their progenies, we classify genotypes into two categories: genotypes with homozygous alleles from Q1028 (designated as a) and genotypes with homozygous alleles from non-Q1028 (designated as b).

The new genetic map on Chromosome 7A was constructed using JoinMap 4 (Van Ooijen 2006). Logarithm of the odds (LOD) ≥3.0 was used to develop the linkage map, and recombination fractions between markers were converted to map distances in cM using the Kosambi mapping function (Fig. 3c).

QTL validation

The flanking markers for a given QTL were used to screen a specific line with or without the allele of this QTL. For example, wPt-5949 and wPt-0961 were flanking markers of *QSd.sau-7A*, the positive allele of which was from Q1028. A line with same genotype with Q1028 for both wPt-5949 and wPt-0961 were regarded as having the allele of *QSd.sau-7A*. Neither QZ13 nor ZM9023 has the allele of *QSd.sau-5A2* or *QSd.sau-7A*. QZ2 has the *QSd.sau-7A* allele only.

Thirty to one hundred lines randomly selected from each of the three validation populations were scored for spike density for validation of the QTL *QSd-sau-7A*, respectively. Based on the presence or absence of marker alleles from the donor parent 'Q1028', the selected lines from each of the three populations were grouped into two classes. The difference in spike density between the two groups within each of the populations was used for identifying the efficiency of the developed markers and measuring the QTL effects. Student's t-test ($P < 0.05$) was used to calculate the differences in spike density between the two groups of alleles.

Results and discussion

Of the 541 genes, 43 were isolated to detect possible polymorphism between Q1028 and ZM9023 (Supplementary Table S1). To detect sufficient polymorphic nucleotides, the conserved core genes among Triticeae were ignored for further isolation. This was performed through searching a given gene in NCBI (http://www.ncbi.nlm.nih.gov/) by selecting species of Triticeae and those genes with varied sequences were selected. Based on our previous study (Ma et al. 2015), the genes with homeologous sequences simultaneously in the 7A, 7B and 7D subgenome were chosen to design chromosome 7A specific primers. Further, the sequences (1~3kbp) located at 5' upstream of start codon and 3' downstream of stop

codon were preferentially isolated as non-coding sequences are usually more polymorphic than coding sequences.

A total of 22 SNPs and 1 indel in 6 genes were detected (Supplementary Table S2). Based on the SNPs, 23 HRM markers were developed (Supplementary Table S3) and tested by Q1028 and ZM9023 and some of their progenies. Finally, three markers (0C32-715, DCE-464, and C81A-240) were proved feasible (Table 1). Although HRM deriving from the combination of existing techniques of DNA melting analysis with a new generation of fluorescent dsDNA dyes (Wittwer et al. 2003) is sensitive and specific for the detection of SNP in PCR products from genomic DNA, we here only successfully developed three markers. It was reported that fluorescent dye (Pirulli et al. 2000; Lipsky et al. 2001; Dufresne et al. 2006), instrument resolution (Herrmann et al. 2006), amplified fragment length (Farrar and Wittwer 2017) could affect the accuracy of HRM analysis. However, these factors could be ruled out given the highly sensitive dye Super EvaGreen, the high resolution analyzer and appropriate amplified fragment (~100-120 bp) applied in this study. The most likely reason for the low efficiency of development of HRM markers is that the primers are not homoeoallele-specific that non-target chromosome fragments were amplified thus interfering the HRM analysis. As reported previously, for polyploidy species, it was difficult to design homoeoallele-specific primers and nested PCR could be employed into HRM analysis (Botticella et al. 2011).

Table 1. Details of the developed HRM markers

Gene	SNP (Q1028/ ZM9023)	Forward primer (5'-3')	Reverse primer(5'-3')	Product length (bp)	Annealing temp.(°C)
Traes_7AL_0C3298060.1	C/T	GTTTGTTCCCTCCACCTAA	GCTTGAACTATC AAAGTGACAT	101	59.1
Traes_7AL_C81A9E290.11	A/G	CTTAATAGCGTACCCTA TGAGATGC	GAGAGACAAGGAC TGACGAAATG	103	60.4
Traes_7AL_DCEBE6FD7.1	T/C	CACGGCAGAAGCATCA CATT	ACGCAGTTCAGAAA GGTAGGA	111	57.9

QZ population were further genotyped using the three HRM markers and integrated genetic map were constructed. Based on the genotype of the 7 markers close to *QSd.sau-7A*, 20 of the RILs were assigned to 6 distinct recombination groups represented as graphical genotypes in Fig. 5a. As shown in Fig. 5b, the spike density of all the groups with marker 0C32-715 having the Q1028-like allele was significantly higher than the group with 0C32-715 having the ZM9023-like allele. Thus, the target QTL *QSd.sau-7A* was speculatively mapped to a 15.3 cM interval between 0C32-715 and wPT-0961 on wheat chromosome 7A (Fig. 3c).

The three newly developed HRM markers were further applied in three validation populations. The marker C81A-240 could not detect polymorphism in any of the three populations. The reason is most likely that this marker is genetically far away from *QSd.sau-7A*. The marker DCE-464 could distinguish all of the three populations. For QZ2×ZM9023 F_2 and QZ2×QZ13 F_2 populations, the average spike density of genotypes with homozygous alleles from Q1028 was significantly higher ($p< 0.01$) than that of genotypes with homozygous alleles from non-Q1028 parents. However, for QE RIL population, no significant difference ($p> 0.05$) of spike density could be detected between the two classes (Table 2). Like QZ population where multiple spike density genes exist, we speculate that the cultivated line 99E18 may also contain genes controlling spike density. Thus, the classified groups by DCE-464 marker linked with *QSd.sau-7A* only could be interfered by other spike density genes, leading to the unreal difference. The maker 0C32-715 could identify polymorphism in QZ2×ZM9023 F_2 only and significant difference between the genotyped groups was also detected. Given the detection ability of the three developed markers, DCE-464 is genetically closer to *QSd.sau-7A* than 0C32-715, and C81A-240 is farthest from *QSd.sau-7A*.

In conclusion, the *QSd.sau-7A* was narrowed down to a 15.3 cM interval between 0C32-715 and wPT-0961 on wheat chromosome 7A. DCE-464 and

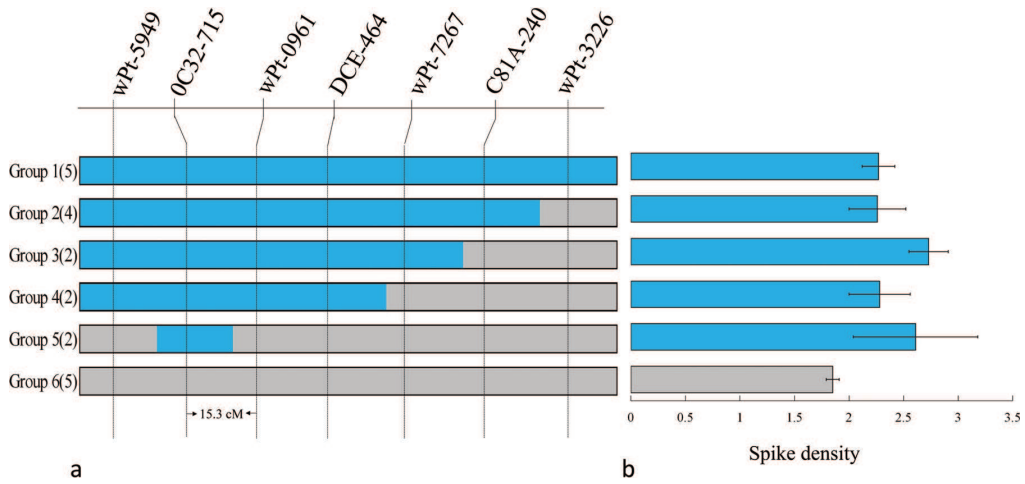

Fig. 5. Spike density maps to a 15.3 cM interval on wheat chromosome 7A, a=Graphical genotypes of Q1028×ZM9023 recombinant inbred line (RIL) groups with the number of lines in each group shown in parentheses. RILs were grouped based on their genotypes defined by having either the Q1028-like (blue) or the ZM9023-like (grey) allele at each marker shown across the interval. b=ANOVA adjusted mean spike density of RIL groups across all experiments. Bars are colored based on a Q1028 - or ZM9023-like phenotype. Blue, Q1028-like; grey, ZM9023-like. Error bars represent SEM

Table 2. Validation of developed markers

Marker	Population	Genotype	Mean (n/cm)	P alue
0C32-715	QZ2×ZM9023 F_2	a	3.432	0.002
		b	2.674	
DCE-464	QZ2×ZM9023 F_2	a	3.150	0.008
		b	2.726	
DCE-464	QZ2×QZ13 F_2	a	3.432	0.006
		b	2.674	
DCE-464	Q1028×99E18 RILs	a	2.038	0.182
		b	1.913	

a=Genotypes with homozygous alleles from Q1028; b=Genotypes with homozygous alleles from non-Q1028

0C32-715 were the most closely linked markers to *QSd.sau-7A*. These two markers could be useful in development of near-isogenic lines and screening of recombinants.

Authors' contribution

Conceptualization of research (JM, XJL); Designing of the experiments (JM); Contribution of experimental materials (XJL); Execution of field/lab experiments and data collection (MS, CCY, NNQ, HZ, PD, YM, HPT); Analysis of data and interpretation (MS, CCY); Preparation of manuscript (JM, MS).

Declaration

The authors declare no conflict of interest.

Acknowledgements

This work is supported by the Key Projects of Education Department of Sichuan Province (16ZA0038), the National Natural Science Foundation of China (31601292 and 31570335), the International Science and Technology Cooperation and Exchanges Program of Science and Technology Department of Sichuan Province (2017HH0076), and the Applied Basic Research Programs of Science and Technology Department of Sichuan Province (2016JY0010).

References

Amagai Y., Kuboyama T. and Watanabe N. 2016. Microsatellite mapping of the gene conferring compact spike in Japanese "Gumbai" landraces of common wheat (*Triticum aestivum* L.). Euphytica, **209**: 709-714.

Botticella E., Sestili, F., Hernandez-Lopez A. et al. 2011. High resolution melting analysis for the detection of EMS induced mutations in wheat *Sbella* genes. BMC Plant Biol., **11**: 156.

Dufresne S., Belloni D., Wells W. and Tsongalis, G. 2006. *BRCA1* and *BRCA2* mutation screening using SmartCycler II high-resolution melt curve analysis. Arch Pathol Lab Med., **130**: 185-187.

Faris J. and Gill B. 2002. Genomic targeting and high-resolution mapping of the domestication gene Q in wheat. Genome, **45**: 706-718.

Faris J., Fellers J, Brooks S. and Gill B. 2003. A bacterial artificial chromosome contig spanning the major domestication locus Q in wheat and identification of

a candidate gene. Genetics, **164**: 311-321.

Farrar J. and Wittwer C. 2017. High-resolution melting curve analysis for molecular diagnostics. In Molecular Diagnostics (Third Edition), pp. 79-102.

Herrmann M., Durtschi J., Bromley L. et al. 2006. Amplicon DNA melting analysis for mutation scanning and genotyping: cross-platform comparison of instruments and dyes. Clin Chem., **52**: 494-503.

International Barley Genome Sequencing Consortium. 2012. A physical, genetic and functional sequence assembly of the barley genome. Nature, **491**: 711.

Jantasuriyarat C., Vales M., Watson C. J. W. and Riera-Lizarazu O. 2004. Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). Theor. Appl. Genet., **108**: 261-273.

Jiang Y., Lan X., Luo W. et al. 2014. Genome-wide quantitative trait locus mapping identifies multiple major loci for brittle rachis and threshability in Tibetan semi-wild wheat (*Triticum aestivum* ssp. *tibetanum* Shao). PLoS One, **9**.

Jiang Y., Wang J, Luo W. et al. 2015. Quantitative trait locus mapping for seed dormancy in different post-ripening stages in a Tibetan semi-wild wheat (*Triticum aestivum* ssp. *tibetanum* Shao). Euphytica, **203**: 557-567.

Johnson E., Nalam V., Zemetra R. and Riera-Lizarazu O. 2008. Mapping the compactum locus in wheat (*Triticum aestivum* L.) and its relationship to other spike morphology genes of the Triticeae. Euphytica, **163**: 193-201.

Kosuge K., Watanabe N., Melnik V., Laikova L. and Goncharov N. 2012. New sources of compact spike morphology determined by the genes on chromosome 5A in hexaploid wheat. Genetic Resour. Crop Ev., **59**: 1115-1124.

Lipsky R., Mazzanti C., Rudolph J.et al. 2001. DNA melting analysis for detection of single nucleotide polymorphisms. Clin. Chem., **47**: 635-644.

Liu Y., Tao Y., Wang Z. et al. 2018. Identification of QTL for flag leaf length in common wheat and their pleiotropic effects. Mol Breed., **38**: 11.

Luo W., Ma J., Zhou X. et al. 2016. Identification of quantitative trait loci controlling agronomic traits indicates breeding potential of Tibetan semiwild wheat *(Triticum aestivum* ssp. *tibetanum*). Crop Sci., **56**:2410-2420.

Ma J., Stiller J., Zheng Z. et al. 2015. A high-throughput pipeline for detecting locus-specific polymorphism in hexaploid wheat (*Triticum aestivum* L.). Plant Methods, **11**: 39.

Ma Z., Zhao, D., Zhang C. et al. 2007. Molecular genetic analysis of five spike-related traits in wheat using RIL and immortalized F_2 populations. Mol. Genet. Genomics, **277**: 31-42.

Mascher M., Gundlach H., Himmelbach A. et al. 2017. A chromosome conformation capture ordered sequence of the barley genome. Nature, **544**: 427.

Mengistu N., Baenziger P., Eskridge K. et al. 2012. Validation of QTL for grain yield-related traits on wheat chromosome 3A using recombinant inbred chromosome lines. Crop Sci., **52**: 1622-1632.

Murray M. and Thompson W. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., **8**: 4321-4326.

Pirulli D., Boniotto M., Puzzer D.et al. 2000. Flexibility of melting temperature assay for rapid detection of insertions, deletions, and single-point mutations of the *AGXT* gene responsible for type 1 primary hyperoxaluria. Clin Chem., **46**: 1842-1844.

RAO M. P. 1977. Mapping of the Sphaerococcum gene '*S*' on chromosome 3D of wheat. Cereal Res Commun, **5**: 15-17.

Shao Q., Li C. and Basang C. 1980. Semiwild wheat from Tibet. Acta Genet Sin., **7**: 149-156.

Simons K., Fellers J., Trick H. et al. 2006. Molecular characterization of the major wheat domestication gene *Q*. Genetics, **172**: 547-555.

Sormacheva I., Golovnina K., Vavilova V. et al. 2015. *Q* gene variability in wheat species with different spike morphology. Genet. Resour. Crop Ev., **62**: 837-852.

Sun Q., Ni Z., Liu, Z. et al. (1998). Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica, **99**: 205-211.

Van Ooijen JW. 2006. JoinMap 4.0, software for the calculation of genetic linkage maps in experimental populations. Kyazama BV, Wageningen.

Wang Z., Liu Y., Shi H. et al. 2016. Identification and validation of novel low-tiller number QTL in common wheat. Theor. Appl. Genet., **129**: 603-612.

Wittwer C., Reed G., Gundry C. et al. 2003. High-resolution genotyping by amplicon melting analysis using LCGreen. Clin. Chem., **49**: 853-860.

Zhang X. and Gao Y. 2004. To design PCR primers with Oligo 6 and Primer Premier 5. China J. Bioinformatics, **2**:14-18

Zhou H., Liu S., Liu Y. et al. 2016a. Mapping and validation of major quantitative trait loci for kernel length in wild barley (*Hordeum vulgare* ssp. *spontaneum*). BMC Genet., **17**: 130.

Zhou X., Ma J., Luo W. et al. 2016b. QTL Mapping for traits of kernel morphology for Tibetan semi-wild wheat. J. Triticeae Crops, **36**: 27-35 .

Zhou Y., Conway B., Miller D. et al. 2017. Quantitative trait loci mapping for spike characteristics in hexaploid wheat. Plant Genome, **10**.

Supplementary Table S1. Primer details for the 43 selected genes

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Length (bp)	locations of primers
Traes_7AL_014D9E18A.1	CAGGTATTGAGCATCGAGCCCT	AAAATTGTTTACGCCCTATTAAA	1319	Promoter region
	CACTTTCAGGGTTTACGTCG	AATTGTTTACGCCCTATTAAAT	1350	Promoter region
Traes_7AL_05785A85F.6	AGAAAGGGAAGGGCAATTTAGAGG	TTGAAGCGTGGGTTTTCCAACTC	981	Promoter region
Traes_7AL_09C024A66.1	AACGGAGGCTCTGCACGAC	AAGCTGATTAGAGAGGTGCCAG	642	Promoter region
	TATATGCAAGATGTTATCAAC	ATATTCCCATGCATCCTACAGGCA	701	Promoter region
	TGCACGACTTCCTGTGCATGTT	GCTGACTGCCTCATCAGCTGA	788	Coding region
	ATTGGCATCCAAGCTTGCCA	TAGAATCAACGGTATTGGGT	650	Coding region
Traes_7AL_0A85A179C.1	GTCGCCACCATGTTTTTAACT	CTGCATCCTGCAGTTCCAGCCA	1043	Promoter region
Traes_7AL_0AC3A4753.5	GTTCACTGAAGCAGTTTTCCC	CATCATCTAGCAACATTTAG	1537	Promoter region
Traes_7AL_0C3298060.1	TTCCATAGGTCTCAATCCTGC	CTTTGGGGGTCATGGAAGCC	814	Promoter region
Traes_7AL_0C45D8295.1	TAAGGTGGATCGGCTCATCG	GTAATGATACGACGACCTAGAG	646	Promoter region
Traes_7AL_0F704586B.1	GTGCTTGCAACTCTACTAAT	GTGAAGGCTATCCAAGGGTA	1537	Promoter region
Traes_7AL_10C0DE3B0.1	CTCGGGCTCGTCGGCGCGAAC	GCAGAGGTACAAGGATGCAT	614	Promoter region
Traes_7AL_10D566B34.1	TCTATGATCTTTCTCGGTGC	ATTTCTCAAGGATCTATGCTTCC	942	Promoter region
Traes_7AL_13DE4FF55.9	GCTGGCTCCGCCCGTTTTGGCAT	GATCACCTCCATAGCTAGCTATA	962	Promoter region
Traes_7AL_158E31A67.7	TATTGAGAGTAGCATTATG	CAATAATAGTACAGATAACCGC	1355	Promoter region
Traes_7AL_199EA4BA2.1	AGGGTCATCACAACAGTGGTGAAG	CGAAGTTGACACTAGCACGGCTA	1493	Promoter region
Traes_7AL_199EA4BA2.1	TGAGGGTCATCACAACAGTGG	CGGAATCGTGTACCTAAAAAC	1205	Promoter region
Traes_7AL_1ADFCB2CD.1	CTCAGATCAATGGATCTCTAGG	CTGATGCATCAAGTTGAGCCAG	854	Promoter region
Traes_7AL_1AE4B1087.1	TTAGTTCGGATTATAAACATAAG	CTGTACGACATTTTCATCAATTGC	1322	Promoter region
Traes_7AL_1E264B402.1	CTGTTATTATCATACTACGG	GTGCGCGCCGTCCGCGTGGTTC	928	Promoter region
Traes_7AL_20DD351C2.1	TTCTTCCGTTGGCTCTGCTTTC	GCGCCGCGGCGCAGAACGGGGC	1438	Promoter region
Traes_7AL_21EB0F3FA.1	CCGACGGCGGCCGTGTAGCAG	CACGCAGAGGTGGTTGTAATCA	894	Promoter region
Traes_7AL_2328547FB.1	CTGTGAAAGTTATATGCAG	CTGCAACTGATAATAAATAATAA	1203	Promoter region
Traes_7AL_233FD66F1.1	TCTCCACCTCCCACCATCCATCG	CAAAAACGAAAAAAGAAAACC	1228	Promoter region
Traes_7AL_2379F24C4.2	GGGCGGCGGTCGAGCGCGTCG	CTATCACTTAATTATAGACGTC	1290	Promoter region
Traes_7AL_28B931610.4	TCCTCGGCCGGCGAGAAGATGCGC	CTTTCCAGTGTTGTTTTGTCGCA	588	Promoter region
Traes_7AL_28D2DE898.4	TACTAGAATAAAATTTGGAGTC	GACTCCGACGCCGAGCCGCCCA	1845	Promoter region
Traes_7AL_2B74F643C.4	CATCGTAGCATGTTACGCAAAC	CCAAGCACAGTTTATTTTTCAAG	1500	Promoter region

Traes_7AL_2C088C7C61.1	TGCCGTGGCCCGGGCCGCAGATG	CTTCCCCCTCTAACCTCGTTTA	1236	Promoter region
Traes_7AL_30F5D9448.7	TCATTCACATCTGAATGCGAAC	ATAACAAATTATTGTAAAAAAAAG	1575	Promoter region
Traes_7AL_31B287764.1	AATCGACCAGCGGCTGGAAC	CCGGATGCGCGAGAGGGCTTG	651	Promoter region
Traes_7AL_32B645073.4	AGCACAAGACATACGAAAAG	CAAAATATAGAGGGTGTTGT	1412	Promoter region
Traes_7AL_32E43BE3F.1	ATCTCCAAAAGTTCATCATCATCC	CGGAGGCCCATCGAACGACAAC	1023	Promoter region
Traes_7AL_33A778289.2	GAACAACAACAGCAATAAAAG	CTAATCCGAAATCAAGAACCATT	1218	Promoter region
Traes_7AL_35165D4BE.1	TGTAACGTCATAAAGGCCGTCG	GTCTCTATGTGGCCATCCTCCA	1365	Promoter region
Traes_7AL_35DF3FD42.1	TCCTCGGGGAGGACGACGACGTG	CGACTTTTTCATTCATTTACTTC	815	Promoter region
Traes_7AL_35E9A3E91.4	TCTTTCTTGAATAAAAGCAAATG	CGCTCATTCTTCACCAGTCTC	1432	Promoter region
Traes_7AL_367C15C93.1	ATGCATGTCAGACACTGTCATAG	CTAGGTGCGCGGTGTTGAGCC	1098	Promoter region
Traes_7AL_8B355A5AB.1	ACTATTGCTATTAAGTCT	TATTGTTTTGAGTAGCTAGCT	774	Coding region
	GATTTTTAAACTAATGGTGT	TATTAAGTGTAATATTTTAA	734	Coding region
Traes_7AL_A647529F4.1	TCGCCGGACGGACGGAAGCA	GCAATACTTTTTGTGCGATC	728	Promoter region
	GGAATTTGATCAGTGGGCTG	CACAAAAAAGTAATCAATTC	634	Coding region
Traes_7AL_BF6FD97DA.1	GCACAGATATGTCTCTCAGCTA	TATCAATGAAAAGCTGCTCCATCA	480	Promoter region
	GATGTATAGGTCAAGCTCT	TAGTTACTCCAACCTTCTCTGG	379	Coding region
	AGAGGGTTCGAGAGGAGAGA	GGCGAGACCCATGCCCGGCA	698	Coding region
Traes_7AL_C81A9E290.1	CGGTAGCCTCCTCGCTCAA	TATCAAGTCTGCAAGATGAT	905	Coding region
	TTAGTCCTATTTGCTTCCTG	TGCCAGAGCTCGAAACTGTG	650	Coding region
	TGCTATCTCCAGACGAACATC	GATGTAGTCTGCAGCTTATCT	639	Promoter region
Traes_7AL_CAE523DB1.1	AGTTGGTAAGTCGTAGTTGGAA	TTCCAACTACGACTTACCAACTT	651	Coding region
	TCTACGAGTACATGGCCAAA	TAATGCAGCGCGCGCATTAA	741	Coding region
Traes_7AL_CD676390F.1	ATGCCAACTATATCTAAG	GCAAGGAGTTCCTTCAGAAGCG	713	Promoter region
	GTAAGCATACTTTAGCACAAT	GTCGGTTGTCACAACAGAAC	694	Promoter region
	GAAGATACCTTATGTTCTAT	TGGATCATAACTTAAAGCAG	712	Coding region
Traes_7AL_DCEBE6FD7.1	CGACCTGCCGGACGTGTACACG	CACTTTCACCTGATCATTCT	530	Promoter region
	GAAGCAGAATCTCCAGAAG	ATAGAATACAGTTAACTGAAC	666	Promoter region
	GGAGGAGAGAGCGCGGCGCTG	GCCTGATCTTGGCTCCACTTG	642	Promoter region
	CGACCTGCCGGACGTGTACACG	CACTTTCACCTGATCATTCT	463	Coding region
Traes_7AL_ECB0591CC.1	GGCTCCGAGTACAGCGCCGC	CGGGCCGCCGACTTCAAGCGCC	346	Promoter region
	CAACTTTACTTGTCTGCCTA	CTGCAGTTAGATATTTGAGT	683	Coding region

Supplementary Table S2. Detected polymorphsim between Q1028 and ZM9023

Gene	Position (bp)	Parent	Sequence near the polymorphsim
Traes_7AL_C81A9E290.11	460	Q1028	ATGAGTGGGAATT**T**CAGACACTTTCAA
		ZM9023	ATGAGTGGGAATT**C**CAGACACTTTCAA
	240	Q1028	TAGGGAGATATCC**A**GAGAATGTTTCCA
		ZM9023	TAGGGAGATATCC**G**GAGAATGTTTCCA
	318	Q1028	GATCCTTCCGGAC**C**CTGCATACAAATC
		ZM9023	GATCCTTCCGGAC**A**CTGCATACCAATC
	327	Q1028	GGACCCTGCATAC**A**AATCCCAACAAGC
		ZM9023	GGACACTGCATAC**C**AATCCCAACAAGC
Traes_7AL_0C3298060.1	614	Q1028	AGCTATGAGCAAC**CAAA**ACAATTAGAA
		ZM9023	AGCTATGAGCAAC**....**ACAATTAGAA
	715	Q1028	CTTAAACTTTGAC**C**GGAACATGTCACT
		ZM9023	CTTAAACTTTGAC**T**GGAACATGTCACT
Traes_7AL_DCEBE6FD7.1	447	Q1028	TGCCGCCTTCGCT**T**GGGCCGGCCCCGG
		ZM9023	TGCCGCCTTCGCT**C**GGGCCGGCCCCGG
	344	Q1028	TGCAAACTGTGGT**A**AGGCTGTTGCTGG
		ZM9023	TGCAAACTGTGGT**G**AGGCTGTTGCTGG
Traes_7AL_CD676390F.1	85	Q1028	ATGTTGCTTCTAG**C**GATTCTGGATCAT
		ZM9023	ATGTTGCTTCTAG**T**GATTCTGGATCAT
	230	Q1028	ATTAAAAAGATTG**T**TAGTGCTTCAGGA
		ZM9023	ATTAAAAAGATTG**C**TAGTGCTTCAGGA
	272	Q1028	GAGCAATATTACT**T**GCTGTGGCGGTGA
		ZM9023	GAGCAATATTACT**G**TCTGTGGCGGTGA
	273	Q1028	AGCAATATTACTT**G**CTGTGGCGGTGAT
		ZM9023	AGCAATATTACTG**T**CTGTGGCGGTGAT
	673	Q1028	GGATTGTGTCAAA**G**TCAAGCCAGCAGA
		ZM9023	GGATTGTGTCAAA**A**TCAAGCCAGCAGA
	755	Q1028	AGAAATTGCACCA**A**TACCAAAGGCGGT
		ZM9023	AGAAATTGCACCA**G**TACCAAAGGCGGT
	823	Q1028	ATGACTTACGAGT**G**ACATACTATATAT
		ZM9023	ATGACTTACGAGT**C**GCATACTATATAT
	824	Q1028	TGACTTACGAGTG**A**CATACTATATATT
		ZM9023	TGACTTACGAGTC**G**CATACTATATATT
	841	Q1028	ACTATATATTTTA**C**GAGCAAGCTATCA
		ZM9023	ACTATATATTTTA**T**GAGCAAGCTATCA
	855	Q1028	GAGCAAGCTATCA**G**GTCGGCGGATTGC
		ZM9023	GAGCAAGCTATCA**C**GTCGGCGGATTGC
Traes_7AL_0F704586B.1	571	Q1028	ATATACTTCCTGT**T**AATGAAATGTATG
		ZM9023	ATATACTTCCTGT**C**AATGAAATGTATG
	281	Q1028	CATACATTTCATT**A**ACAGGAAGTATAT
		ZM9023	CATACATTTCATT**G**ACAGGAAGTATAT
Traes_7AL_014D9E18A.1	400	Q1028	GAAATAGTGATTT**C**TGACTTCCAGTAC
		ZM9023	GAAATAGTGATTT**T**TGACTTCCAGTAC
	11	Q1028	AAAGAAATCAC**C**TAAGTAGGTGC
		ZM9023	AAAGAAATCAC**A**TAAGTAGGTGC
	470	Q1028	ACGATTCACACTT**C**TTCTCCATTTCAC
		ZM9023	ACGATTCACACTT**T**TTCTCCATTTCAC

Supplementary Table S3. HRM markers development information

Gene	Maeker name	Marker sequence (5'-3')	Parent	Sequence close to the SNPs
Traes_7AL_C81A9E290.11	C81A-460F1	GCTGTAATTCCTCTGCCTCAC	Q1028	ATGAGTGGGAATT**T**CAGACACTTTCAA
	C81A-460R1	CGGTGCTGCTTTGATCTTGTA	ZM9023	ATGAGTGGGAATT**C**CAGACACTTTCAA
	C81A-460F2	GCCAGCAACACAGCAGTA	Q1028	ATGAGTGGGAATT**T**CAGACACTTTCAA
	C81A-460R2	ACGATGGGGAGAAAAGAGGTA	ZM9023	ATGAGTGGGAATT**C**CAGACACTTTCAA
	C81A-240F1	CTTAATAGCGTACCCTATGAGATGC	Q1028	TAGGGAGATATCC**A**GAGAATGTTTCCA
	C81A-240R1	GAGAGACAAGGACTGACGAAATG	ZM9023	TAGGGAGATATCC**G**GAGAATGTTTCCA
	C81A-240F2	TCTTAATAGCGTACCCTATGAGATG	Q1028	TAGGGAGATATCC**A**GAGAATGTTTCCA
	C81A-240R2	AACTATGAGAGACAAGGACTGAC	ZM9023	TAGGGAGATATCC**G**GAGAATGTTTCCA
Traes_7AL_0C3298060.1	0C32-715F1	GCTCCAACTTGCTGTTTG	Q1028	CTTAAACTTTGAC**C**GGAACATGTCACT
	0C32-715R1	GCTTGAACTATCAAAGTGACAT	ZM9023	CTTAAACTTTGAC**T**GGAACATGTCACT
	0C32-715F2	GTTTGTTCCCTCCACCTAA	Q1028	CTTAAACTTTGAC**C**GGAACATGTCACT
	0C32-715R2	GCTTGAACTATCAAAGTGACAT	ZM9023	CTTAAACTTTGAC**T**GGAACATGTCACT
Traes_7AL_DCEBE6FD7.1	2DCE-446F1	ACGGCAGAAGCATCACATT	Q1028	TGCCGCCTTCGCT**T**GGGCCGGCCCCGG
	2DCE-446R1	ACACGCAGTTCAGAAAGGTAG	ZM9023	TGCCGCCTTCGCT**C**GGGCCGGCCCCGG
	2DCE-447F2	CACGGCAGAAGCATCACATT	Q1028	TGCCGCCTTCGCT**T**GGGCCGGCCCCGG
	2DCE-447R2	ACGCAGTTCAGAAAGGTAGGA	ZM9023	TGCCGCCTTCGCT**C**GGGCCGGCCCCGG
Traes_7AL_CD676390F.1	CD6-85F1	GCAGGCTAATGAGATTGAAGGAT	Q1028	ATGTTGCTTCTAG**C**GATTCTGGATCAT
	CD6-85R1	AGACTGCCGTGTACTTCCATA	ZM9023	ATGTTGCTTCTAG**T**GATTCTGGATCAT
	CD6-85F2	CAGGCTAATGAGATTGAAGGA	Q1028	ATGTTGCTTCTAG**C**GATTCTGGATCAT
	CD6-85R2	ACCAGGTTGATTCTTTCTGAA	ZM9023	ATGTTGCTTCTAG**T**GATTCTGGATCAT
	CD6-841F1	CGGTAAGCATATTTTAGCACAATCC	Q1028	ACTATATATTTTA**C**GAGCAAGCTATCA
	CD6-841R1	CAATCCGCCGACGTGATAG	ZM9023	ACTATATATTTTA**T**GAGCAAGCTATCA
	CD6-841F2	GCGGTAAGCATATTTTAGCACAATC	Q1028	ACTATATATTTTA**C**GAGCAAGCTATCA
	CD6-841R2	GCAATCCGCCGACGTGATA	ZM9023	ACTATATATTTTA**T**GAGCAAGCTATCA
	CD6-673F1	CAGGACAATGGATGCAGTAGC	Q1028	GGATTGTGTCAAA**G**TCAAGCCAGCAGA
	CD6-673R1	CAGAAGCGGGTCCAATAATAGTG	ZM9023	GGATTGTGTCAAA**A**TCAAGCCAGCAGA
	CD6-673-F2	CCAGGACAATGGATGCAGTAG	Q1028	GGATTGTGTCAAA**G**TCAAGCCAGCAGA
	CD6-673-R2	TTCAGAAGCGGGTCCAATAATAG	ZM9023	GGATTGTGTCAAA**A**TCAAGCCAGCAGA
	CD6-755F1	AGCCAGCAGACTCCATCT	Q1028	AGAAATTGCACCA**A**TACCAAAGGCGGT

	CD6-755R1	AGGATTGTGCTAAAATATGCTTACC	ZM9023	AGAAATTGCACCA**G**TACCAAAGGCGGT
	CD6-755F2	AATCAAGCCAGCAGACTCCAT	Q1028	AGAAATTGCACCA**A**TACCAAAGGCGGT
	CD6-755R2	GTGCTAAAATATGCTTACCGCCTT	ZM9023	AGAAATTGCACCA**G**TACCAAAGGCGGT
Traes_7AL_0F704586B.1	0F7-571F1	GTGTAACGGGGTTTCCTTTCTTTT	Q1028	ATATACTTCCTGT**T**AATGAAATGTATG
	0F7-571R1	CGATGAACCAGGGCATGAAC	ZM9023	ATATACTTCCTGT**C**AATGAAATGTATG
	0F7-571F2	AACGGGGTTTCCTTTCTTTT	Q1028	ATATACTTCCTGT**T**AATGAAATGTATG
	0F7-571R2	GTAGATTGGCCTCGATGAAC	ZM9023	ATATACTTCCTGT**C**AATGAAATGTATG
	0F7-281F1	CGATGAACCAGGGCATGAAC	Q1028	CATACATTTCATT**A**ACAGGAAGTATAT
	0F7-281R1	GTGTAACGGGGTTTCCTTTCTTTT	ZM9023	CATACATTTCATT**G**ACAGGAAGTATAT
	0F7-281F2	CGATGAACCAGGGCATGAACA	Q1028	CATACATTTCATT**A**ACAGGAAGTATAT
	0F7-281R2	GTGTAACGGGGTTTCCTTTCTTTT	ZM9023	CATACATTTCATT**G**ACAGGAAGTATAT
Traes_7AL_014D9E18A.1	014-400F1	CCCAAGAAAGAAAGAAAGCAAGCAA	Q1028	GAAATAGTGATTT**C**TGACTTCCAGTAC
	014-400R1	TGGAGAAGAAGTGTGAATCGTTGG	ZM9023	GAAATAGTGATTT**T**TGACTTCCAGTAC
	014-400F2	CCCAAGAAAGAAAGAAAGCAAGCAA	Q1028	GAAATAGTGATTT**C**TGACTTCCAGTAC
	014-400R2	ATGGAGAAGAAGTGTGAATCGTT	ZM9023	GAAATAGTGATTT**T**TGACTTCCAGTAC
	014-400F1	GGTGGTCCCAAGAAAGAAAGAAA	Q1028	GAAATAGTGATTT**C**TGACTTCCAGTAC
	014-400R1	AAGAAGTGTGAATCGTTGGC	ZM9023	GAAATAGTGATTT**T**TGACTTCCAGTAC