Identification and map-based cloning a new gene TaSP1 related to spike shape by EMS-induced wheat mutant

Lin Zhang
Henan University

Huidan Zhou
Henan University

Xian Fu
Henan University

Niuniu Zhou
Henan University

Mengjie Liu
Henan University

Shenglong Bai
Henan University

Xinpeng Zhao
Henan University

Ruiru Cheng
Henan University  https://orcid.org/0000-0002-1253-665X

Suoping Li
Henan University

Dale Zhang
zhangdale97@126.com

Henan University  https://orcid.org/0000-0002-6504-7999

Research Article

Keywords: Wheat, Spike shape, mutant, TaSP1, cloning

Posted Date: February 20th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3944811/v1
Version of Record: A version of this preprint was published at Theoretical and Applied Genetics on May 6th, 2024. See the published version at https://doi.org/10.1007/s00122-024-04621-0.
Abstract

Spike shape, an important factor for wheat grain yield, is mainly defined by spike length (SPL), spikelet number (SPN) and compactness. ZM1160, a mutant obtained from ethyl methane sulfonate (EMS) treatment of hexaploid wheat variety Zhoumai32, was used to identify and clone the candidate gene conditioned the spike shape. Genetic analysis of an F$_2$ population derived from a cross of ZM1160 and Bainong207 suggested that the compact spike shape in ZM1160 was controlled by a single recessive gene, and therefore the mutated gene was designated as $Tasp1$. With polymorphic markers identified through bulked segregant analysis (BSA), the gene was mapped to a 2.65 cM interval flanked by markers YZU0852 and MIS46239 on chromosome 7D, corresponding to a 0.42 Mb physical interval of Chinese spring (CS) reference sequences (RefSeq v1.0). To fine map $TaSP1$, 15 and 7 recombinants were respectively screened from 1599 and 1903 F$_3$ plants derived from the heterozygous F$_2$ plants. Finally, $TaSP1$ was delimited to a 21.9 Kb (4,870,562 to 4,892,493 bp) $Xmis48123-Xmis48104$ interval. Only one high-confidence gene $TraesCS7D02G010200$ was annotated in this region, which encodes an unknown protein with a putative vWA domain. The quantitative reverse transcription PCR (qRT-PCR) analysis showed that $TraesCS7D02G010200$ was mainly expressed in the spike. Haplotype analysis of 655 wheat cultivars using the candidate gene-specific marker G010200P2 identified a superior haplotype $TaSP1b$ with longer spike. $TaSP1$ is beneficial to the improvement of wheat spike shape.

Key message

A candidate gene $TaSP1$ related to spike shape was cloned, and the gene-specific marker was developed to efficiently track the superior haplotype in common wheat.

Introduction

As one of the three main cereal crops worldwide, common wheat ($Triticum aestivum$ L., 2$n = 6x = 42$, AABBDD), provides more than 20% of the calories consumed by the human population (Gao 2021). With the rapid growth of human population, wheat global production still needs to be enhanced to meet the increasing demand in the coming decades (https://www.fao.org/faostat/en/#data/QCL). Improving wheat yield per unit is an important way to solve this problem. Grain yield is a complex agronomic trait controlled by multiple genes, and is affected by three major components: thousand-grain weight (TGW), grain number per spike (GNS), and spike number per unit area (Wang et al. 2018; Zhang et al. 2022). Wheat spike morphology is mainly characterized by spikelet number (SPN), spike length (SPL) and spike compactness (SC), which is closely related to TGW and GNS. Therefore, genetic improvement of wheat spike traits is an effective way to achieve high grain yield (Panda et al. 2020).

The multiple sessile spikelets of wheat interlacing along the central spike axis can directly develop into compound spike, and the development is a complex biological process influenced by multiple genetic and physiological factors (Gao et al. 2019). Wheat spike differentiation development could be divided into three consecutive steps: spike meristem initiation and development, spikelet meristem development
and floret meristem development. A few genes related to these stages have been identified. Vernalization (VRN), Photoperiod (Ppd), Flowering locus (FT), and domestication gene Q all regulate the spike meristem initiation and development stage (Yan et al. 2004, 2006; Fu et al. 2005; Beales et al. 2007; Li et al. 2008; Zhang et al. 2011; Kippes et al. 2014; Boden et al. 2015; Greenwood et al. 2017; Dixon et al. 2018; Zhang et al. 2019). At the spikelet meristem development stage, WHEAT FRIZZY PANICLE (WFZP) in AP2 gene family, SQUAMOSA promoter binding protein-like 14 (TaSPL14), AGAMOUS-LIKE6-like (TaAGL6), and WHEAT ABERRANT PANICLE ORGANIZATION 1 (WAP01) are mainly functioned (Dobrovolskaya et al. 2014; Cao et al. 2021; Kong et al. 2022; Kuzay et al. 2022). At the last stage, crucial genes, such as Grain Number Increase1 (GNI1), SQUAMOSA and SHORT VEGETATIVE PHASE (SVP), regulate the meristem development of floret (Sakuma et al. 2013). The interaction between SQUAMOSA and SVP can promote the terminal spikelet formation and stem elongation in early reproductive stage of wheat (Li et al. 2021a). Recently, TaCOL-B5 encoding a CONSTANS-like protein, a gene increased spikelet nodes number per spike and produced more tillers and spikes was cloned in emmer wheat (Zhang et al. 2022).

Wheat spike morphology traits, SPL, SPN and SC, are all controlled by polygenes. Many studies have paid attention to identify the quantitative trait loci (QTL) conditioned these traits. To date, a large number of QTLs for SPL, SPN and SC, distributing on most wheat chromosomes, have been identified by linkage mapping or genome-wide association study (Ma et al. 2007; Gao et al. 2015; Guo et al. 2018; Li et al. 2018; Li et al. 2021b; Ma et al. 2018; Ma et al. 2019; Si et al. 2023). Specifically, QTLs with major effects or detected in multiple populations on SPL have been found on chromosomes 1A, 2D, 3A, 3D, 4A, 4B, 5A, 6A, 6B, 7A, 7B and 7D; and those associated with SPN on 1B, 2A, 2D, 3D, 5B, 7A and 7D (Ma et al. 2019; Ding et al. 2022; Si et al. 2023) and those associated with SC on 2D, 5A and 6A (Hu et al. 2023; Li et al. 2021b). However, few QTLs have been validated and fine-mapped.

It is an effective approach to identify the QTLs/genes of the target trait based on the mutant induced by EMS (Abe et al. 2012; Takagi et al. 2015; Deng et al. 2019; Zhou et al. 2020). In this study, we characterized a compact spike shape mutant ZM1160 from an EMS mutagenized population of common wheat variety Zhoumai32. The objectives were to (1) identify and clone the candidate gene related to the spike shape, and (2) develop the candidate gene-specific markers efficiently tracking the superior haplotype in common wheat.

**Materials and Methods**

**Plant materials**

The spike shape mutant line ZM1160 was isolated from screening of a mutant library of hexaploid wheat Zhoumai32, which was created and preserved in the Plant Germplasm Resources and Genetic Engineering Laboratory, Henan University. A mapping population of 190 F2 plants, generated from crossing ZM1160 and Bainong207 (with normal spike shape), and the corresponding F2:3 families were used for the preliminary mapping of TaSP1. About 3502 F3 plants derived from the heterozygous F2
individuals were used for fine mapping and map-based cloning of TaSP1. In addition, 655 common wheat accessions from China were randomly selected and used to determine the haplotype of the TaSP1 candidate gene-specific marker (G010200P2) (Supplementary Table 1).

**Phenotypic evaluation and field management**

The mapping populations along with the parents were grown in the wheat breeding farm of Kaifeng for phenotypic evaluation in the 2020–2021 crop season. Twenty seeds were planted in 1.5-m rows with an inter-row space of 0.25 m. About 30 to 35 seeds from each F$_{2:3}$ families were sown and evaluated the genotype of the corresponding F$_2$ individual under controlled chamber conditions with 18 h light, and a 24°C /20°C day/night cycle. The spike shapes and flag leaf length were investigated at the heading time. At maturity, about 10 plants from the middle of each row were harvested for evaluating 100-grain weight, grain length, grain width, spike length, spikelet number per spike. The force required for breaking apart the rachis at the flowering stage was measured as newton (N) with the plant stem strength tester YYD-1 (Zhejiang topu yunnong Technology Co., Zhejiang, China). Ten rachises were measured for both wild type and the mutant.

Four hundred and three and 252 common wheat varieties were respectively planted in the wheat breeding farm of Xinxiang and Kaifeng in the 2022–2023 crop season. Each plot included two 1.5 m rows spaced by 0.25 m. Fifteen seeds per row were evenly planted. Spike length (SL) was surveyed in both trails at maturity stage. For each plot, SL were represented as the mean of ten plants randomly chosen from the middle of the rows.

**Bulked segregant analysis**

Two DNA pools were made separately with thirty mutant and thirty normal spike F$_2$ plants. The ZM1160 and Bainong207 parents were sampled from 10 plants. The genomic DNA was extracted using a standard CTAB method (Xue et al. 2022). The two DNA pools, together with the two parents were detected and genotyped using the Axiom® wheat 660K SNP array (Thermo), which was performed by China Golden Marker (Beijing) Biotech Co., Ltd. (CGMB, http://www.cgmb.com.cn/). High quality genotyping data were obtained by filtering with the Dish QC threshold of > 0.82 and the Call-Rate threshold of > 94.

The two DNA pools and the recessive parent ZM1160 were re-sequenced to discovery more SNPs in the target interval. In detail, 1 µg DNA per sample was fragmented by sonication to an average size of 300–400 bp. The libraries containing selected fragments were sequenced using a BGISEQ-500 platform with a paired-end read length of 150 bp. The raw data were filtered by using SOAPnuke (Chen et al. 2018) to obtain clean reads with sequencing depths of more than 30 × for each accession. The remaining high-quality reads were mapped to the CS reference sequences (RefSeq v1.0, IWGSC 2018) by using the Burrows-Wheeler Alignment tool (BWA, Li and Durbin 2009). The duplicated reads were marked and removed using the Genome Analysis Toolkit (GATK, McKenna et al. 2010). The SNPs were determined
with the GATK HaplotypeCaller module, and their positions were located based on the CS reference sequences (RefSeq v1.0).

**Genotyping**

Based on the re-sequencing results of the two DNA pools, SNPs in the target interval were used to design cleaved amplified polymorphic sequences (CAPS) or degenerate the derived cleaved amplified polymorphic sequences (dCAPS) markers. All primers were designed with Primer Premier 5.0 (Premier Biosoft International, USA). Primer sequences of the polymorphic markers were listed in Supplementary Table 1. The markers were amplified in a T100™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) according to the following procedures: 94°C for 3 min; 35 cycles of 94°C for 30 s, 52–60°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. The polymerase chain reaction (PCR) profile contained 5 µl of 2× Taq PCR master Mix II (TIANGEN, Beijing, China), 0.5 µl of primer mix (10 µM for each primer), 2 µl (100–200 ng) of genomic DNA, and 2 µl of ddH₂O. The PCR products were analyzed by electrophoresis with 8% non-denaturing polyacrylamide gels.

**Cloning and qRT-PCR**

The target gene was cloned and sequenced according to Li et al. (2017). The DNA sequencing was performed by Invitrogen (Beijing, China). The final nucleotide sequences for the target gene were determined from three independent clones. The ORF of the candidate gene was translated into amino acid sequence using the ORF Finder program at the NCBI (http://www.ncbi.nlm.nih.gov). The alignment of sequences was carried out using the multiple sequence alignment software Clustal X 2.0 (Larkin et al. 2007). The conserved domains of target gene were predicted by the SMART website (https://smart.embl.de).

Total RNA was extracted with the RNAprep Pure Plant Plus Kit (Tiangen, Beijing, China) following the manufacturer’s instructions. For gene expression assays, the root at seedling stage, stem at booting stage, young spikes at double-ridge stage, terminal spikelet stage and different length, and flag leaf at heading stage, were respectively collected. Each sample contained three biological replicates. The first-strand cDNA was synthesized from 1.5 µg total RNA using a reverse transcription kit FastKing RT Kit (Tiangen, Beijing, China). The qRT-PCR reactions of three technical replicates were performed with primer G010200QP3 using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) following the previously described procedure (Long et al. 2018). The TaGAPDH gene was used as an internal control, and the 2^–ΔΔCt method was used to calculate the relative gene expression (Livak and Schmittgen 2001). All primers for gene cloning and qRT-PCR assays are listed in Supplementary Table 1.

**Statistical analysis**

Chi-squared (χ²) tests were performed to test the goodness-of-fit. The genetic linkage map was constructed with QTL IciMapping v4.0 (Meng et al. 2015) using the maximum likelihood algorithm and the Kosambi function. The significant differences of spike length between two independent samples obtained by haplotype analysis were evaluated by Nonparameter test (Mann-Whitney U).
Results

Characterization of mutant ZM1160

We characterized a compact-spike mutant, ZM1160, from an EMS mutagenized population of the wheat variety Zhoumai32. Spikes and awns of the mutant were much shorter than the wild type (Fig. 1a, c). In the mutant, the spikelets, florets and seeds displayed an irregular shape (Fig. 1b, 1d-g); the spike rachis was more fragile (Fig. 1k). Moreover, the traits of the mutant, such as spike length, grain weight, awn length, grain length, grain width, and flag leaf length showed statistically significant differences from the wild type (Fig. 1h, j; Supplementary Fig. 1). The spikelet number per spike did not change significantly (Fig. 1i).

Inheritance of the malformation spike shape in ZM1160

To assess inheritance of the compact spike shape, we conducted a cross, ZM1160 × Bannong207. F₁ plants from the cross showed normal spike shape (Fig. 2). Of 190 derived F₂ population, 147 displayed normal spike shape, the remaining were consistent with ZM1160, fitting a 3:1 ratio ($\chi^2 = 0.327, P = 0.05$). Progeny test with the F₂ plants yielded 57 lines with mutant spike, 90 lines showing spike shape segregation, 43 lines with normal spikes, conforming to the 1:2:1 Mendelian ratio ($\chi^2 = 1.179, P = 0.05$). These results suggested that the mutant spike shape was controlled by a single recessive gene, therefore the mutated gene was designated as $Tasp1$.

TaSP1 fine mapping

The BSA pools with compact and normal spike shapes along with the two parents were genotyped with the Wheat 660K SNP Array. A total of 391 SNPs were identified between the two parents as well as the pools, of which 343 were clustered in a 15.85 Mb genomic region (1.03–16.88 Mb) on chromosome 7DS (Supplementary Fig. 2), indicating that the $TaSP1$ gene is most likely located on chromosome 7DS. The dCAPS markers located in the target region were developed and subsequently detected for polymorphisms. Linkage mapping with 190 F₂ plants and six polymorphic markers YZU0693, YZU0852, YZU1006, YZU1296, YZU1636, and MIS46239 initially mapped $TaSP1$ to a 2.65 cM genetic interval flanked by $Xyzu0852$ and $Xmis46239$, corresponding to a 0.42 Mb physical interval (Supplementary Fig. 3, Supplementary Table 2).

To further narrow down the region of $TaSP1$ gene, the two DNA pools and the recessive parent ZM1160 were re-sequenced to identify more SNPs in the target interval. Seven polymorphic dCAPS markers were developed based on the SNPs resided in the 0.42 Mb physical interval. A total of 15 recombinant individuals representing 13 genotypes in the $Xyzu0852$-$Xmis46239$ interval were identified from 1599 F₃ individuals derived from the heterozygous F₂ plants (Supplementary Table 3). Based on the genotypes and spike shape of the recombinants, the $TaSP1$ was fine-mapped to the interval encompassed by $Xmis46203$ and $Xmis46234$ with a physical distance of 198 kb. Seven recombinants were identified from
another 1903 F\textsuperscript{3} plants with markers MIS46203 and MIS46234. The 10 recombinants covered \textit{Xmis46203-Xmis46234} interval then were genotyped with 4 newly-designed polymorphic markers, and they could be divided into 6 types (Supplementary Table 4). Finally, \textit{TaSP1} was mapped to a 22.9 Kb (4,870,562 to 4,892,493 bp) interval flanked by \textit{Xmis48123} and \textit{Xmis48104}, in which only one highly confident gene \textit{TraesCS7D02G010200} was annotated (Fig. 3).

Sequencing of \textit{TraesCS7D02G010200} from ZM1160, Zhoumai32 and Bainong207 revealed that two SNPs in the coding sequences (Fig. 4a, b). The first SNP, C in wild type Zhoumai 32 and Bainong207 while T in mutant ZM1160, was the mutant one. The other SNP at 1449 bp was identified between Zhoumai32 and Bainong207. Two markers, MIS48109 and G010200P2, were developed based on these two SNPs, and they co-segregated with \textit{Tasp1} in 190 F\textsubscript{2} plants and recombinants (Supplementary Fig. 4; Supplementary Table 4).

### Candidate gene analysis

The full gene sequences of \textit{TraesCS7D02G010200} from wild type and mutant were cloned and sequenced, and no intron was found by comparing its full-length cDNA with genome sequence. The gene \textit{TraesCS7D02G010200} encodes an unknown protein with a putative vWA domain. The C-to-T transition at 1138 bp in the ORF generated a non-synonymous change R340W in the putative von Willebrand factor A (vWA) domain (Fig. 4c).

To investigate expression pattern of \textit{TraesCS7D02G010200}, transcription levels in root tips, stem, flag leaf, and spikes were respectively examined. As shown in Fig. 5a, the gene \textit{TraesCS7D02G010200} was mainly expressed in the spike, expression level of young spike (~ 2 cm in length) was about 90-fold that of the stem. Moreover, during the spike development process, \textit{TraesCS7D02G010200} was barely expressed at double-ridge stage and terminal spikelet stage, and the expression levels increased first and then decreased along with the rachi elongation process (Fig. 5b).

### Allelic analysis of the \textit{TraesCS7D02G010200} in common wheat cultivars

The gene \textit{TraesCS7D02G010200} was cloned and sequenced from randomly selected cultivars, Aikang58, Zhoumai18, Zhoumai28, Zhengmai7698, Zhengmai366, Xinmai26, and CS. Only one SNP (C/G) consistent with that between Zhoumai32 and Bainong207 was found at 1449 bp among their full-ORF sequences (Supplementary Fig. 5). Therefore, \textit{TaSP1} haplotypes in 655 cultivars (403 planted at Xinxiang, 253 planted at Kaifeng) were examined using the candidate gene-specific marker G010200P2 for the SNP (C/G) (Supplementary Fig. 6). All these wheat varieties could be divided into two haplotypes, \textit{TaSP1}a (Bainong207 type) and \textit{TaSP1}b (Zhoumai32 type). The frequency of \textit{TaSP1}a was much higher than that of \textit{TaSP1}b (Fig. 6a). Compared with \textit{TaSP1}a, \textit{TaSP1}b has longer spike length (Fig. 6b, c). These results indicate that there is still a great potential to utilize \textit{TaSP1} for wheat spike genetic improvement.

### Discussion
Understanding the development and regulation of inflorescences is of great importance to increase grain production in flowering plants. In this study, we characterized the compact spike mutant ZM1160 derived from bread wheat Zhoumai32. This recessive mutation, *Tasp1*, was mapped on chromosome 7DS and co-segregated with *Xmis48109*. We showed that *TaSP1* candidate gene encodes an unknown protein with a putative vWA domain. The missense mutation, the SNP (C/T) detected at 1138 bp in the vWA domain might result in the malformation spike shape.

*TaSP1* was mapped to the telomere on chromosome 7DS. Some intervals associated with spike traits on this chromosome have been reported. For example, Li et al. (2021b) identified a stable QTsn.cib-7D controlled spikelet number per spike on chromosome 7D in five environments by the Kechengmai1/Chuanmai42 doubled haploid (DH) population. An environmentally stable QTL affecting SPL and SC was mapped to an interval near the centromere of chromosome 7D (Xu et al. 2022). Chen et al. (2020a) reported that QTspn.cau-7D located at 60–90 Mb on chromosome 7D was associated with total spikelet number per spike. None of these QTLs overlap with the position of *TaSP1*. QTgw.cau-7D, regulating grain width and weight, was delimited to the 4.4 Mb physical interval at the downstream of *TaSP1* (Chen et al. 2020b). qSL7D.1, a QTL associated with spike length, was identified to the 3.79–10.73 Mb interval on the chromosome 7DS in a RIL population derived from an early heading mutant (eh1) and Lunxuan987 (Xiong et al. 2021). Analysis of the physical position indicated that *TaSP1* might be the causal gene for qSL7D.1.

*TaSP1* pleiotropically regulated SPL, SC, flag leaf, rachis fragility, awn and grain morphology. Unlike *Q*, mutation of *TaSP1* resulted in short, compact and fragile spike, which indicated that *TaSP1* may participate in different regulatory roles from *Q*. Since the *Tasp1* mutant showed similar to *Tasg-D1* mutant, which both of them exhibited a shorter and more compact spike, shorter awns, smaller spikelets and grains, wider and shorter flag leaf (Cheng et al. 2020), how they interact in determining development of these traits is certainly interesting. Most of the genes related wheat spike development, like *TaAGL6*, *Q*, *WFZP*, *GNI1* and *WAPO*, are reported to be transcription factors (Dobrovolskaya et al. 2015; Kong et al. 2022; Kuzay et al. 2022; Sakuma et al. 2019; Zhang et al. 2011). However, the gene identified here encodes an unknown protein with a putative vWA domain, and the subcellular location prediction showed the gene was not localized in nuclear (data not shown), which indicated *Tasp1* was not a transcription factor. Besides, *TraesCS7D02G010200* was mainly expressed at the rachi elongation stage not the spike differentiation stage, which also differed from these previously reported genes. Our study broadened the view of the mechanism underlying wheat spike development.

The vWA domain primarily involved in protein-protein interaction in multiprotein complexes is well characterized in humans (Karkute et al. 2022). However, vWA domain-containing proteins in plants are least explored. In *Arabidopsis thaliana* and rice, several copine genes which contains vWA domain are identified to function in defense response, bacterial and fungal disease (Katkute et al. 2022). Recently, leaf rust resistance gene *Lr9* encodes an unusual tandem kinase fusion protein containing vWA domain in wheat was cloned (Wang et al. 2023). All of these reported genes are functioned in biotic stress resistance, how the vWA domain-containing protein affect wheat development has not been elucidated.
yet. Our further work on the mechanisms of *TaSP1* will shed light on the emerging role of vWA domain-containing proteins in wheat spike development. Moreover, the candidate gene-specific marker G010200P2 developed here can be used in molecular marker-assisted selection for the improvement of wheat spike shape.

**Declarations**

**Acknowledgements**

This work was partially supported by Key Technology R&D Program of Henan Province of China (225200810024, 231111112900), Natural Science Foundation of Henan Province of China (232300421155), Key Technology Program of Henan Province of China (222103810013, 222102110138).

**Conflicts of interest/Competing interests**

The authors declare that they have no conflicts of interest.

**Ethics approval**

Not applicable

**Consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and material**

Available upon request.

**Code availability**

Not applicable

**Authors' contributions**

LZ conducted fine mapping and data analysis; HZ, XF, NZ and ML participated in field trials and data collection; SB and XZ conducted bioinformatic analysis; SL and DZ constructed the population and supervised the project; RC and DZ performed data analysis and wrote the article.

**References**


Figures
Figure 1

Phenotypes of the wild type Zhoumai32 (left) and mutant ZM1160 (right). **a**, spikes at flowering stage; **b**, spikelet; **c**, spike rachis; **d**, floral; e-f, grain morphology; h-k, spike length (h), spikelet number (i), hundred-grain weight (j), and rachis strength (k) of Zhoumai32 and ZM1160. ** and *** indicate significant differences at $P = 0.01$ and $P = 0.001$ respectively. Bar= 1cm (a-d), 5mm (e-g).
Figure 2

Spikes of Bainong207, ZM1160 and their filial generations. Scale bar, 1 cm.
Figure 3

Map-based cloning of *TaSP1*. *TaSP1* was narrowed down to an ~21.9 kb region of chromosome 7D containing one ORF. The markers and numbers of recombinants are indicated.
**Figure 4**

*TaSP1* nucleotide sequences and protein structures of Bainong207, Zhoumai32 and ZM1160. Protein structures were predicted by the SMART website. The blue boxes indicated the mutant nucleotide; the mutant amino acids were highlighted with the red line.
Figure 5

Expression pattern of *TaSP1* in Zhoumai32. **a** *TaSP1* relative expression level in various tissues. The RNA samples were collected from roots in wheat seedling stage, flag leaves during wheat heading period, stems and young spikes during wheat booting stage. **b** *TaSP1* expression in spikes at different developmental stages. DR, TS, young spikes at double ridge stage and terminal spikelet stage; S2, S3, …, and S9 represent young spikes at 2 cm, 3 cm, …, and 9 cm in length, respectively.

Figure 6

Phenotypic effects of the two alleles *TaSP1*a and *TaSP1*b on spike length in common wheat cultivars. **a** Haplotype frequencies of *TaSP1* in detected wheat varieties cultivated at Xinxiang and Kaifeng. **b, c**
Spike length comparison of *TaSP1a* and *TaSP1b* in Xinxiang (b) and Kaifeng (c). * indicates significant difference at *P*=0.05.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.xlsx
- SupplementaryMaterialfiguresnew.docx
- ElectronicSupplementaryInformation.docx