An Overexpressed Q Allele Leads to Increased Spike Density and Improved Processing Quality in Common Wheat (Triticum aestivum)

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ABSTRACT Spike density and processing quality are important traits in modern wheat production and are controlled by multiple gene loci. The associated genes have been intensively studied and new discoveries have been constantly reported during the past few decades. However, no gene playing a significant role in the development of these two traits has been identified. In the current study, a common wheat mutant with extremely compact spikes and good processing quality was isolated and characterized. A new allele (Qc1) of the Q gene (an important domestication gene) responsible for the mutant phenotype was cloned, and the molecular mechanism for the mutant phenotype was studied. Results revealed that Qc1 originated from a point mutation that interferes with the miRNA172-directed cleavage of Q transcripts, leading to its over-expression. It also reduces the longitudinal cell size of rachises, resulting in an increased spike density. Furthermore, Qc1 increases the number of vascular bundles, which suggests a higher efficiency in the transportation of assimilates in the spikes of the mutant than that of wild type. This accounts for the improved processing quality. The effects of Qc1 on spike density and wheat processing quality were confirmed by analyzing nine common wheat mutants possessing four different Qc alleles. These results deepen our understanding of the key roles of Q gene, and provide new insights for the potential application of Qc alleles in wheat quality breeding.

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MicroRNAs (miRNAs) are ~22 nucleotides in length RNAs that repress the expression of genes post-transcriptionally, mainly by DNA elimination, mRNA cleavage, and translational repression (Mallory and Vaucheret 2006). Currently, increasing data demonstrate that miRNAs play critical functions in almost all biological and metabolic processes in plants (Sun 2012). One well studied example is miRNA172, which regulates floral organ identity, flowering time, spike density, and stress response. An accumulation of miRNA172 in Arabidopsis results in early flowering, and disrupts floral organ identity (Aukerman and Sakai 2003), with defects in carpels and a reduction in stamen number (Chen 2004; Zhao et al. 2007). The regulation
between miRNA172 and its targets, SCHLAFMÜTZE (SMZ) and SCHNARCHZAPFEN (SNZ) (Schmid et al. 2003), represses flowering. Overexpression of miRNA172 in rice causes lower fertility and reduced seed weight (Zhu et al. 2009). In barley, the elongation of inflorescence internodes is affected by miRNA172-HvAP2 regulation, which results in an extreme spike density (Houston et al. 2013). Overexpression of RAP2.1, which possess the miRNA172 target region, leads to greater sensitivity to cold and drought stress in Arabidopsis (Dong and Liu 2010). A transgenic Arabidopsis line of soybean miRNA172 shows tolerance to salt stress and increased sensitivity to ABA by regulating its target gene (Li et al. 2015). In wheat, a miRNA172-AP2-like system plays a crucial role in regulating of flowering time, and in spike morphogenesis (Debernardi et al. 2017). Overexpression of miRNA172 leads to an elongated spike (Debernardi et al. 2017; Liu et al. 2017).

Processing quality is a valuable trait in wheat breeding and production. Diverse food has been developed to take advantage of the unique properties of wheat flour (i.e., mixing characteristics, dough rheology, and baking performance). Gluten, including high molecular weight glutenin subunit (HMW-GS), low molecular weight glutenin subunit (LMW-GS) and gliadins (Payne 1987), and genes regulating the expression of gluten affect wheat processing quality. SPA (Storage Protein Activator), homologous to the Opaque2 gene in maize, is a key regulator of the expression of gluten in wheat (Ravel et al. 2009). A NAC (NAM, ATAF, and CUC transcription factor) gene can increase grain protein content (GPC) in wheat (Uauy et al. 2006). DOF (DNA binding with one finger) can activate the expression of α-gliadin genes during grain filling (Dong et al. 2007).

Although the genes/gene loci associated with spike density and processing quality have been intensively studied, no gene playing a significant role in the development of these two traits was identified. In the current study, a common wheat mutant, with increased spike density and improved processing quality, was isolated. We demonstrated that a point mutation within the miRNA172-binding site of Q gene altered its transcriptional level, which is responsible for the mutant phenotype. This research deepens our understanding of the Q gene in wheat development, and provides new insights for the potential utilization of the Q gene in wheat.

**MATERIALS AND METHODS**

**Wheat materials and growth conditions**

A common wheat mutant (S-Cp1-1) with increased spike density was isolated from 0.6% ethyl mesylate (EMS)-treated common wheat (Triticum aestivum L.) cultivar “Shumai482.” S-Cp1-1 and its corresponding wild type (WT) used were isolated from an M₀ heterozygous plant. Nine independent mutants related to S-Cp1-1 (Supplemental Material, Table S1 in File S1) were obtained from 0.8% EMS-treated T. aestivum cv “Shumai482,” “Liangmai4,” “Mianmai37,” and “Roblin,” respectively. S-Cp1-1 was used as the female parent in crossing with shumai482 (a hexaploid wheat line) and wanke421 (a common wheat cultivar), to construct segregation populations. The plants were grown at the experimental farm of Sichuan Agricultural University in Wenjiang, with row spacing of 20 × 10 cm. A nitrogen: phosphorous: potassium (15: 15: 15; 450 kg per hectare) compound fertilizer was used before sowing.

To assess the effect of mutant gene on processing quality, S-Cp1-1 and its WT were grown at the experimental farm in Wenjiang as well, in a randomized block design with three replications for two growing seasons (2014–2015 and 2015–2016). Each replicate was planted with an area of 2 × 4 m, with row spacing of 20 × 5 cm. A nitrogen: phosphorous: potassium (15: 15: 15; 450 kg per hectare) compound fertilizer was used before sowing.

**Figure 1** Phenotype of S-Cp1-1 and mapping of the Cp1 locus. (A) Plants of S-Cp1-1 (left) and WT (right) at GS29. (B) Plants of S-Cp1-1 (left) and WT (right) at GS59. (C) Spikes of S-Cp1-1 (left) and WT (right) at GS59 (D) Rachises of S-Cp1-1 (left) and WT (right) at GS59. The rachises between white arrows were used in Figure 6. (E) Stem and spike lengths of S-Cp1-1 and WT at GS90. Data are means ± SD (SD; n = 35). **P** < 0.01. (F) Mapping of the Cp1 locus. The BACs (bacterial artificial chromosomes) and genomic scaffolds were queried using a BLASTN algorithm in NCBI (http://www.ncbi.nlm.nih.gov/) and aligned based on their relative positions and overlap. All of the BACs and scaffolds used are listed in Table S3 in File S1. Scale bar (A–D), 1 cm.

**Microscopy analysis**

The developing spikes of S-Cp1-1 and WT were scanned using an optical microscope (Olympus, Tokyo, Japan) and EPSON perfection V700 (EPSON, Tokyo, Japan). The spikes at GS59 (decimal code of wheat development; Zadoks et al. 1974) were fixed in FAA solution (70% alcohol; 37% formaldehyde; acetic acid = 18: 1: 1, v: v: v) and embedded in paraffin. Then, the paraffin wax was cut into 6-µm sections using a Leica slicer (Leica, Wetzlar, Germany). Safranin O/fast green (Solarbio, Beijing, China) was used for staining. The splices were photographed by using a BX60 light microscope (Olympus).
Genetic mapping and gene cloning

Genomic DNA was extracted from the young leaves of F2 individuals derived from S-Cp1-1·br220 (Doyle and Doyle 1987). Genomic DNAs of 10 randomly selected F2 individuals with compact spike/normal spike were pooled, and 24 DNA pools were constructed. The pooled DNA samples were analyzed by Illumina 90K single nucleotide polymorphism (SNP) microarray at Compass Biotechnology (Beijing, China), to primarily locate the mutant locus. The locus was then mapped by sequence-tagged site (STS) and simple sequence repeat (SSR) markers in an F2 population containing 819 plants, derived from S-Cp1-1·br220. STS and SSR markers (Table S2 in File S1) were developed based on the SNP markers and the physical map draft of Triticum urartu (Ling et al. 2013; http://plants.ensemble.org/index.html; http://www.gramene.org/gremene/searches/ssrtool). The genetic linkage map was constructed according to the method described by Jiang et al. (2014).

The cDNA and genomic DNA sequences of candidate gene were PCR amplified from both mutant and WT plants, and confirmed by Sanger sequencing (Invitrogen, Shanghai, China). Sequences were analyzed by DNAMAN V6 (Lynnon Biosoft, San Ramon). The primers used are listed in Table S2 in File S1.

RNA extraction and qRT-PCR analysis

The spikes of S-Cp1-1 and its WT counterpart were collected at GS24, GS29, and GS32 (Zadoks et al. 1974). There were three biological replicates for each stage, with at least 10 spikes for each replicate. Root, stem, and leaf samples of S-Cp1-1 and its WT at GS24 stage were collected as well. Three biological replicates were done for each tissue, with 10 plants used per replicate. The harvested samples were ground in liquid nitrogen, and RNA was extracted using the Plant RNA extraction kit V1.5 (Biot, Chengdu, China).

qRT-PCR reactions were performed using a SYBR premix Ex Taq RT-PCR kit (Takara, Dalian, China). RNA clean up, cDNA synthesis, qRT-PCR analyses were performed as described in Wang et al. (2010). Two housekeeping genes (Long et al. 2010), i.e., Scaffold-associated regions (SAR) DNA binding protein (NCBI UniGene Ta.14126) and methionine aminopeptidase 1 (Ta.7894), were amplified as reference genes.

The seeds were harvested in two growing seasons, i.e., 2014–2015 and 2015–2016. “A” and “B” represent significance at P < 0.01. Significance was calculated by using t-test and LSD test. E, environment; G, genotype.

| Table 1 Comparison of processing quality parameters of S-Cp1-1 to its WT |
|------------------|------------------|------------------|------------------|------------------|
|                  | GPC (%) | WGC (%) | Zeleny Sedimentation Value (ml) | Development Time (min) |
|                  | 2014–2015 | 2015–2016 | 2014–2015 | 2015–2016 | 2014–2015 | 2015–2016 | 2014–2015 | 2015–2016 |
| S-Cp1-1          | 19.72A   | 17.90A   | 50.60A    | 41.95A    | 63.05A    | 45.87A    | 7.11A    | 3.23A     |
| WT               | 14.00B   | 11.49B   | 34.83B    | 20.09B    | 36.83B    | 20.09B    | 2.46B    | 1.41B     |
| F value          | E       | <0.01    | 221.15    | <0.01    | 51.67     | <0.01    | 295.41   | <0.01     |
| P value          | G       | <0.01    | 574.10    | <0.01    | 110.61    | <0.01    | 360.68   | <0.01     |
| F × G E          | G × E   | 0.041    | 15.12     | <0.01    | 0.73      | 0.417    | 55.27    | <0.01     |

The seeds were harvested in two growing seasons, i.e., 2014–2015 and 2015–2016. "A" and "B" represent significance at P < 0.01. Significance was calculated by using t-test and LSD test. E, environment; G, genotype.

Figure 2

Expression of Qc1 measured by qRT-PCR. (A–D) Spikes of S-Cp1-1 (left) and WT (right) at GS22, GS24, GS29, and GS32, respectively. Scale bar, 0.1 cm (A and B) and 1 cm (C and D). (E) Relative expression levels of Qc1 and Q in root, stem, and leaf at GS24. (F) Relative expression of Qc1 and Q at GS24, GS29, and GS32. Error bars represent means ± SD (n = 3).
Denmark), a zeleny analysis system (CAU-B, Beijing, China), and a 2013, using an automatic azotometer (Kjelec 8400; FOSS, Hillerød, Germany). The grain protein content (dryweight), zeleny sedimentation milled with a Brabender Quandrumat Junior mill (Brabender, Duisburg, Germany). Grain samples were cleaned and adjusted to 14% moisture, before being processing quality analysis.

Figure 3 Genomic structure of Q<sup>2</sup>- and confirmation of miRNA172-directed regulation in the developing spike at GS24. (A) Genomic structure of the Q<sup>2</sup>-. The initiation and termination codons, exons (black rectangles), and introns (gray rectangles) are illustrated. The point mutations in the miRNA172-binding site of q, Q, and Q<sup>2</sup>- are indicated. (B) miRNA172 cleavage sites in the transcripts of Q<sup>2</sup>- and Q as determined by 5’ RACE.

5’ modified RACE
The 5’ rapid amplification of cDNA end (RACE) analysis was performed as in Llave et al. (2002). Total RNA was extracted from spikes of S-Cp1-1 and its WT at the GS24 using a Plant RNA extraction kit V1.5 (Biofit). The primers for the first and second PCR products were Q-cDNA-R and 3’RACE-R (Table S2 in File S1), respectively. The second PCR products were purified and cloned into the pMD19-T vector (Takara) for sequencing.

SDS-PAGE analysis
Seed storage proteins were extracted from 20 mg whole wheat flour and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Qi et al. (2011).

Processing quality analysis
Grain samples were cleaned and adjusted to 14% moisture, before being milled with a Brabender Quadrumat Junior mill (Brabender, Duisburg, Germany). The grain protein content (dry weight), zeleny sedimentation value and wet gluten content were measured following GB/T 17320-2013, using an automatic azotometer (Kjelc 8400; FOSS, Hillerød, Denmark), a zeleny analysis system (CAU-B, Beijing, China), and a glutomatic 2200 system (Perten, Härgersten, Sweden), respectively.

Dough rheological properties were determined with a 10-g Mixograph (TMCO, Lincoln, NE). Samples were mixed to optimum water absorption following the 54-40A method (AACC 2001). The development time (minutes to the curve peak) was measured. Finally, results were collected and analyzed using the MixSmart software.

The baking test was performed according to AACC method 10.09-01 (AACC 2010) with some modifications. The baking procedure was the standard rapid-mix-test with 40 g flour at 14% moisture content. Three biological replicates were conducted with two breads for each flour sample. The loaf volume was determined by BVM6630 volume meter (Pertern) following the manufacturer’s instruction.

Statistical analysis
The chi square (χ<sup>2</sup>) test (for mutant phenotype), t-test (for processing quality), and least-significant difference (LSD) test under a general linear model (for processing quality) were carried out using DPS software (version 12.01; Tang and Zhang 2013).

Data availability
All data necessary for the conclusions are represented in the paper’s tables, figures and supplemental information. The mutants are available upon request. Nucleotide sequence data from this article can be found in the GenBank database under the following accession numbers: KX580301–KX580304 and KX620761–KX620768.

RESULTS
Identification of new Q alleles
A common wheat mutant (S-Cp1-1), with increased spike density (compact spike) and improved processing quality (Figure 1 and Table 1), was isolated. S-Cp1-1 had a similar architecture to its WT before GS30 (decimal code of wheat development; Zadoks et al. 1974; Figure 1A). Thereafter, its plant height was gradually lower than that of the WT (Figure 1B). The spike density of S-Cp1-1 differed from that of WT earlier than GS22 (Figure 2A). The increased spike density and reduced plant height were not separated in the BC<sub>1</sub>F<sub>2</sub> population (1010 individuals), suggesting that they are controlled by the same locus. S-Cp1-1 was used as the female parent in a cross with br220 (a hexaploid wheat line), to develop a mapping population. To facilitate genetic research, the increased spike density was selected as the target trait for mapping. A chi square (χ<sup>2</sup>) test showed that the spike density of 819 F<sub>2</sub> plants (Table S1 in File S1) was followed by a 3:1 segregation ratio (χ<sup>2</sup> = 0.12; P = 0.99), suggesting that the increased spike density in S-Cp1-1 was controlled by a single dominant locus (Cp1).

Cp1 was positioned on 5AL (Figure S1 in File S1, Table S4), by using a wheat 90K SNP microarray and 24 DNA pools of F<sub>2</sub> plants generated...
from S-Cp1-1 × br220. Subsequently, STS and SSR markers (Table S2 in File S1) were developed, Cp1 was placed in a 1.6 cM region between markers qg2 and qg3, with 0.4 and 1.2 cM in the F2 population, respectively Table S5. Notably, the chromosome region flanked by qg2 and qg3 contained the Q gene, whose overexpression increases spike density (Simons et al. 2006). Q is an AP2 transcription factor, containing a miRNA172-binding site in the 10th exon. To demonstrate whether the increased spike density in S-Cp1-1 was caused by Q, we developed an intragenic molecular marker (qg1) for Q gene. qg1 cosegregated with compact spike in 10,100 F3 individuals derived from S-Cp1-1 × br220. One missense mutation (C-T; serine to leucine; Figure 3A and Figure S2 in File S1) in the miRNA172-binding site of S-Cp1-1 was identified (GenBank nos. KX580301 and KX580302). To facilitate the following description, we named the new Q allele as Qc1 (the first Q leading to compact spike).

The effect of Qc1 on spike density was confirmed by analyzing nine independent common wheat mutants possessing four different Q alleles (Qc1-Qc4; Figure 4 and Table S1 in File S1). These alleles contained four different point mutations in the miRNA172-binding site, supporting a causal relationship between the transcriptional regulation of Q by miRNA172 and the mutant phenotype.

### Comparison of expression levels

The transcriptional levels of Qc1 and Q were compared by qRT-PCR. The ratios of transcriptional level of Qc1 to that of Q were 3.8 at GS24 (Figure 2, B and F), 1.9 at GS29 (Figure 2, C and F), and one at GS32 (Figure 2, D and F), respectively. It revealed that the increased spike density in S-Cp1-1 was a result of higher transcriptional level of Qc1 in developing spike before GS32 (Figure 2, A–D). Besides spike, Qc1 and Q expressed differentially at the RNA level in root, stem, and leaf at GS24 as well. Relative to Q, the transcriptional levels of Qc1 were 10.2-fold in root, 9.9-fold in stem, and 3.8-fold in leaf (Figure 2E).

To validate whether the higher transcriptional level of Qc1 was due to altered cleavage directed by miRNA172, 5'-RACE analysis was carried out. The sequencing of 20 randomly chosen Qc1 and Q clones showed that the cleavage site in the miRNA172-binding region was changed. We can conclude that the point mutation in Qc1 disturbed in vivo cleavage by miRNA172 (Figure 3B), suggesting that the phenotype of S-Cp1-1 was due to overexpression of Q, resulting from the point mutation that interferes with the miRNA172-directed cleavage of the Q transcripts.

### Effect of new Q alleles on grain quality

Four parameters reflecting wheat processing quality were compared between S-Cp1-1 and its WT in two growing seasons (Table 1). In contrast to the WT control, GPC, wet gluten content, and development time were significantly higher (P < 0.01) for S-Cp1-1. The average of loaf volume of S-Cp1-1 was 37% greater (P < 0.01) than that of the WT (Figure 5). No variation in the composition of gluten was observed between S-Cp1-1 and WT (Figure 5S in File S1), especially HMW-GS, which is among the most important determinants in bread-making quality (Shevry et al. 2003). To assess the effect of Qc1 on processing quality in different genetic backgrounds, GPC of individual plants belonging to two F2 populations was measured (Table 2). As expected, GPC of plants with two copies of Qc1 allele was significantly higher than those with one or no Qc1 copies (P < 0.01). The effect of Qc1 on processing quality was confirmed by analyzing independent common wheat mutants possessing four different Q alleles (Table S1 in File S1) as well.

### Effect of Qc1 on cells of rachis

A microscopic comparison of the longitudinal sections of rachis indicated that the cells in S-Cp1-1 were decreased in size (Figure 6C) compared with those in the WT (Figure 6D). It is obvious that Qc1 reduced the longitudinal cell size of rachis, resulting in increased spike density in S-Cp1-1. Transverse sections of rachis revealed that cells of S-Cp1-1 were reduced in size and increased in number, and, notably, the number of vascular bundles in S-Cp1-1 was increased (Figure 6, A and B). The increase in the number of vascular bundles suggested a higher efficiency in the transportation of assimilates in the spikes of the mutant than that of WT. This accounts for the improved processing quality of S-Cp1-1. Additionally, the vascular morphology was changed in S-Cp1-1 (Figure 6A). There were a lower number of xylem cells in the vascular bundles, and a greater number of cells around the vessels (Figure 6, A and E) when compared with the WT (Figure 6, B and F).

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**Table 2 Effect of Qc1 on grain protein content (dry weight) in two F2 populations**

| Genotype | GPC (%) |
|----------|---------|
| S-Cp1-1 × Br220 | S-Cp1-1 × wanke421 |
| Qc1/Qc1 | 20.00A | 22.43A |
| Qc1/Q | 14.56B | 17.19B |
| Q/Q | 11.88C | 10.32C |
| F value | P value |
| Population | 46.1 | <0.01 |
| Genotype | 1163.0 | <0.01 |
| P × G | 63.6 | <0.01 |

The seeds of 20 individual F2 plants were harvested for each of the lines with zero, one or two Qc1 copies. “A,” “B” and “C” indicate significance at P < 0.01. Significance was calculated by using t-test and LSD test.
DISCUSSION
During the domestication of common wheat, changes in gross morphology of the spike enhanced its suitability for wheat production. There are three major genes that affect gross morphology of the spike in common wheat, i.e., Q, C, and S1 (Morris and Sears 1967), which have taxonomic importance. The Q gene on chromosome 5AL pleiotropically influences many characters, including spike density and seed threshability (Faris and Gill 2002; Simons et al. 2006). The C gene on chromosome 2D (Johnson et al. 2008) genetically controls the compact spike in a subspecies of hexaploid wheat known as T. aestivum ssp. compactum. The S1 gene on chromosome 3D determines the unique spike morphology of T. aestivum ssp. sphaerococcum (Sears 1947). The results of the current study indicate an effect on spike density similar to that the C gene of a new Q allele, suggesting that there is some similarity between the molecular pathways of these two genes in regulating spike density.

As a major domestication gene in wheat, Q arose through a point mutation occurring in the miRNA172-binding site of q (Figure 3A; Simons et al. 2006). Q’ alleles originated from the introduction of more point mutations into the miRNA172-binding site of Q (Figure 4B). Interestingly, the transcriptional levels of q, Q, and Q’1 are correlated with the number of point mutations in the miRNA172-binding site, indicating that post-transcriptional regulation plays a critical role in the expression of the Q gene (Figure 2, E and F; Simons et al. 2006). Greenwood et al. (2017) reported a relationship between a point mutation in the miRNA172-binding site of Q (equivalent to the Q’2 allele in this paper) and spike density. Here, we identified three new point mutations in the miRNA172-binding site of Q in different genetic backgrounds (Figure 4), further demonstrating that overexpression of Q is the causal mechanism for the observed change in spike morphology in mutants.

For miRNA-directed cleavage, base-pairing between miRNAs and their target mRNAs is critical (Huntzinger and Izaurralde 2011). The most important feature for mRNA–miRNA pairing is the “seed site” (Bartel 2009), which is 2–7 nt from the 5’ region of miRNAs. The point mutations in q and Q’ (Q’2 allele in this paper) (Simons et al. 2006;
Greenwood et al. (2017) occur in the seed site, which induce dramatic phenotype changes. However, what happens if the point mutations occur outside the seed site of miRNA172 binding region of Q gene remains unclear. It was uncertain whether point mutations outside the seed site interfere with the cleavage of Q and ultimately bring about kindred or a new phenotype. We identified three new alleles (Q^{*}, Q^{2}, and Q^{3}) in different genetic backgrounds with nucleotide polymorphisms outside the seed site of the miRNA172 binding region of the Q gene. The mutants with these new alleles exhibit similar phenotypes as that of Q^{2} (Figure 4 and Table S1 in File S1), indicating that the seed site of the miRNA172 binding region within Q gene is not as strict as expected.

Consistent with the results of Liu et al. (2017) and Greenwood et al. (2017), our 5′ RACE analysis show that the point mutation in the miRNA172-binding site can disturb in vivo cleavage by miRNA172, leading to overexpression of the Q gene. Furthermore, inhibition of miRNA172 activity by a miRNA target mimic resulted in compact spikes (Debernardi et al. 2017). Overexpression of bread wheat miRNA172 caused a speltoid-like spike phenotype (Liu et al. 2017). These results point to a critical role of miRNA172 in regulation of the Q gene at the transcript level.

Improvements in wheat processing quality have been studied extensively over the years. However, the effect of Q on wheat processing quality was rarely studied. The unique properties of wheat flour depend primarily on seed storage proteins—one of the most important sources of protein for human beings—which consist mainly of glutenins and gliadins (Payne 1987; Shewry et al. 2003). These latter proteins are responsible for dough elasticity and extensibility. Diverse food has been developed to take advantage of the properties of wheat flour. Despite its significance in human life, efforts to improve the processing quality of wheat have been hindered by a complex genetic system and strong environmental effects (Simmonds 1995). Wheat processing quality is a complex of characteristics controlled by a large number of genes (Ma et al. 2009). GPC is a crucial index for measuring wheat quality (Weegels et al. 1996), and is a frequent target in wheat breeding. The genetic components of GPC in wheat have been extensively studied for many years. The greatest effect was detected by Loppa et al. (1997), who found a QTL explaining 66% of the phenotypic variation of GPC. The identified gene in this QTL encodes a NAC transcription factor, which is associated with a GPC increases of ~1.3 g kg^{-1} (Uayu et al. 2006). In contrast to Q, Q^{*} is correlated with GPC increases of ~60 g kg^{-1} (Table 1), suggesting a key role of Q in regulating the accumulation of seed storage proteins in wheat. It is well known that Q has a profound effect on the spread of polyloid wheat, since, in contrast to the q allele, it allowed early farmers to easily harvest wheat. Considering the significant effect of Q^{*} on GPC and loaf volume (Figure 5 and Table 1), we can speculate that processing quality and nutritional quality might have been important factors for selection of Q by early farmers as well. It will be interesting to compare GPC of wheat near isolines for q, Q, and Q^{*} alleles.

Compared to Q, the Q^{2} allele reduces the longitudinal cell size of rachis, resulting in an increased spike density, which is not a favorable character in most wheat-growing areas. Consistent with the results of Simons et al. (2006), Greenwood et al. (2017) indicated that amino acid replacement in the AP2 domain of Q can decrease spike density. Therefore, it is very hopeful that we will be able to obtain wheat lines with new Q alleles that contribute to processing quality improvement without affecting spike morphology. Liu et al. (2017) suggested the potential role of the bread wheat transcriptional corepresser TOPELESS (TaTPL) in the regulation of spike density. The N-terminal ethylene-responsive element binding factor-associated amphiphilic repression (EAR) (LDLNVE) motif mediates interaction of Q protein with TaTPL. Jost et al. (2016) demonstrated the effect of a homolog of Blade-On-Petiole 1 and 2 (BOP1/2) on internode length and homeotic changes of the barley inflorescence. Determination of the interaction between Q and the known and unknown genes would be helpful to understand the molecular mechanisms on spike density, and thus be helpful to promote the utilization of Q alleles in wheat breeding.

In summary, we characterized a new allele for the Q gene—an important domestication gene—and demonstrated that point mutations in the miRNA172-binding site altered the transcriptional level of Q gene during the development of wheat spike, which contributes to increased spike density and improved processing quality of mutants. These results deepen our understanding of the key roles of the Q gene, and provide new insights for the potential application of Q alleles in wheat quality breeding.

ACKNOWLEDGMENTS
We thank Hong-Yang Yu, Jing Fan and Wen-Ming Wang in Sichuan Agricultural University for their technical assistance. We thank Jose Barrero in CSIRO for suggestions. This research was supported by the National Natural Science Foundation of China (31230053, 31570335, and 31671677), and the National Basic Research Program of China (2014CB147200).

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Communicating editor: E. Akhunov