New alleles of the wheat domestication gene Q reveal multiple roles in growth and reproductive development

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Summary statement:
Novel alleles are used to define new roles for the wheat AP2 gene, Q, in inflorescence architecture, including the formation and structure of specialized grain producing branches known as spikelets.
Abstract

The advantages of free threshing in wheat led to the selection of the domesticated $Q$ allele which is now present in almost all modern wheat varieties. $Q$ and the pre-domestication allele, $q$, encode an AP2 transcription factor with the domesticated allele conferring a free threshing character and a subcompact (i.e. partially compact) inflorescence (spike). We demonstrate that mutations in the miR172-binding site of the $Q$ gene are sufficient to increase transcript levels via a reduction in miRNA dependent degradation, consistent with the conclusion that a SNP in the miRNA-binding site of $Q$ relative to $q$ was essential in defining the modern $Q$ allele. We also describe novel gain- and loss-of-function alleles of $Q$ and use these to define new roles for this gene in spike development. $Q$ is required for the suppression of ‘sham ramification’ and increased $Q$ expression can lead to the formation of ectopic florets and spikelets (specialized inflorescence branches that bear florets and grains) resulting in a deviation from the canonical spike and spikelet structures of domesticated wheat.
Introduction

The causal molecular mechanism for the domestication of Q is thought to be either an amino acid change in the predicted Q protein (Simons et al., 2006), and/or a SNP present in a presumed miRNA-binding site of Q (Sormacheva et al., 2015, Chuck et al., 2007). Unlike the domesticated homeoallele Q (Chromosome 5A)(Faris et al., 2003), the B and D homeoalleles of hexaploid bread wheat are thought to be a pseudogene and lowly expressed, respectively (Zhang et al., 2011). Q is a member of the AP2 class of transcription factors, known to influence many traits associated with floral transition, including both flowering time and the definition of floral organs (Aukerman and Sakai, 2003, Chuck et al., 2007, Lauter et al., 2005, Lee and An, 2012, Lee et al., 2007, Brown and Bregitzer, 2011, Varkonyi-Gasic et al., 2012). Generally, gain-of-function mutations and overexpression of AP2 genes result in delayed flowering (Mlotshwa et al., 2006, Aukerman and Sakai, 2003, Schmid et al., 2003, Jung et al., 2007), and additional florets in the Tassleseed6 mutant of corn (Chuck et al., 2007). Loss-of-function mutations and reduced expression can cause early flowering and disruptions in floral patterning and determinacy (Chuck et al., 2008, Chuck et al., 1998, Lee and An, 2012, Mlotshwa et al., 2006, Jung et al., 2007, Mathieu et al., 2009); these effects can be masked by redundant function of other AP2s (Yant et al., 2010). AP2s can be regulated by miR172, and mutations affecting the expression of miR172 or SNPs in either miR172 or its conserved target site in AP2 genes can lead to misregulation with the potential to increase or reduce regulatory targeting by miR172 (Aukerman and Sakai, 2003, Chuck et al., 2007, Varkonyi-Gasic et al., 2012, Zhu et al., 2009).

Results and Discussion

A dwarf, compact spike mutant was identified in an M2 mutant population derived from the Australian wheat cultivar Sunstate (SS) and backcrossed to the progenitor line, SS. The F2 progeny of this cross could be separated into three distinct height classes, SS-like, intermediate (heterozygous) and short (homozygous mutant) (Fig. 1A,B), which were subsequently confirmed by genetic analysis (see below). Differences in height were marked (Fig. 1B,C) and unambiguously separated plants into the three classes. Both heterozygous and homozygous mutant plants were characterised by a reduction in internode length relative to SS-like siblings resulting in spike compaction and reduced overall height (Figs. 1C,D, S1). In addition, mutant plants were late flowering and possessed a small increase in rachis node number (nodes along the spike that potentially bear a fertile spikelet) (Fig. 1E,F).

The compact mutant resembled transgenic wheat lines with increased copy number and expression of Q (Simons et al., 2006, Förster et al., 2011, Forster et al., 2013). The mutant (hereafter called Q’) contained a novel single nucleotide change in the miRNA-binding site of Q that causes an additional mismatch when aligned to the targeting miRNA, Ta-miR172 (Fig. 2A, S2). No other sequence changes
were observed in the coding region. Expression of Q, as measured by qPCR, was higher in developing inflorescences of Q’ plants compared to their SS-like siblings (Fig. 2B, C). Modified 5’ RACE detected Q cleavage products in mRNA from SS-like plants, while only a single cleavage product was detected from Q’ mRNA (Fig. 2D). The most abundant class of Q cleavage products matched the expected product from miRNA-directed cleavage between the 10th and 11th nucleotide within the miRNA, while the single Q’ cleavage product detected was shifted by a single base. Combined with our expression data, reduced levels of cleavage product in Q’ indicates that the induced mismatch to Ta-miR172 results in reduced targeted mRNA degradation, and ultimately higher Q protein abundance. Based on its partial genetic dominance and increased mRNA expression, Q’ appears to be a gain-of-function allele relative to Q. We cannot exclude the possibility that the amino acid change (G-E) in the predicted Q protein resulting from the Q’ SNP also contributes to the observed phenotypes. However, similar compact spike phenotypes have been reported in transgenic plants containing a miR172-binding site mimic (MIM172) and reduced levels of miR172 (Debernardi et al., 2017 companion paper), suggesting that reduced miR172 cleavage in Q’ is sufficient to induce the observed phenotypes. For the barley paralogue, HvAP2(2H), both synonymous and non-synonymous mutations causing mismatches to miR172 result in compact spikes further supporting that nucleotide mismatches without amino acid changes can affect this trait (Houston et al., 2013).

To confirm that the Q’ mutation was causal, we first confirmed complete genetic linkage between the Q’ SNP and the reduced height/compact spike phenotypes (Fig. 3B). Secondly, we investigated additional alleles. Two allelic dwarf mutant lines with compact spikes, ANBW5C Dwarf (5CD) and ANBW5B Dwarf (5BD) (Fig. 3A), have previously been described and mapped to chromosome 5AL (which contains Q), although the genetic basis was not determined (Kosuge et al., 2011). Sequencing revealed that both mutant lines contained SNPs within the miRNA-binding site of Q with one mutant line, 5CD, containing the exact same mutation as Q’ and the other, 5BD (Q’-like), featuring a unique SNP in the miRNA-binding site of Q (Figs. 3C, S2). The similar phenotypes of the two independent mutants support the hypothesis that the causal effect of the Q’ mutation is associated with a reduction in miR172 repression rather than the change in the encoded amino acid. Consistent with impaired regulation by miR172, all compact spike mutants showed higher relative expression of Q compared to their sibling or parent lines in both developing inflorescence and elongating peduncle internode tissue (Fig. 3G, H).

To formally confirm that the Q’ mutation was causal to the observed phenotypes, we performed a second round of mutagenesis in the Q’ background. Two unique revertant alleles were isolated with SNPs in the first exon of the Q’ gene, one (Q’-Rev1) a presumed complete loss-of-function revertant with an introduced stop codon, and the other (Q’-Rev2) a partial revertant with an amino acid change,
immediately before the first predicted AP2 binding domain (Fig. 3C). Both ‘revertants’ retained the Q and Q’ mutations, as expected. The Q’ revertants completely (Q’-Rev1) or partially (Q’-Rev2) suppressed the observed phenotypic changes in Q’ (see below), confirming that changes in Q are responsible for the Q’ gain-of-function phenotypes.

The presence of independent gain- and loss-of-function Q alleles in a common background allows the function of Q to be analysed with a precision not previously possible. In contrast to Q’, Q’-Rev1 plants showed a reduction in rachis node number compared to SS-like plants demonstrating an earlier (in terms of nodes) transition from inflorescence meristem to terminal spikelet meristem (Fig. 3F). While plant height was increased in Q’-Rev1 plants compared with SS-like plants, spike length was not significantly different (Fig. 3D, E). Reduced rachis node number in Q’-Rev1 compared with Q meant that the average internode length between each spikelet was greater resulting in reduced spikelet density, also known as a lax spike. Compared to SS-like and Q’, the lax spikes of Q’-Rev1 were difficult to hand thresh (Fig. S3) consistent with observations of plants containing pre-domestication q, or 5A deletions that lack domesticated Q (Faris et al., 2003, Simons et al., 2006, Förster et al., 2011), and with Q playing an important role in wheat domestication. Partial reversion of the Q’ mutant phenotype in Q’-Rev2 was characterised by an increase in height and spike length relative to Q’ but not to the extent of SS-like plants (Fig. 3A).

Given that AP2 genes in other species have diverse roles in spikelet and floret development, we sought to determine whether increased Q activity resulted in additional, undescribed changes in reproductive development. The two independent Q’ mutants and Q’-like all exhibited several alterations in spikelet and floret development, although we focused on detailed analysis of the original Q’ allele in the SS background. Q’ plants produced fully formed, floret containing spikelets usually by the second rachis node from the base of the spike whereas Q and Q’-Rev1 plants typically produced 3-4 rudimentary spikelets at the basal rachis nodes before producing fertile floret-bearing spikelets, as often occurs for modern wheat varieties (Figs. 4D, S4). Thus, increased Q activity can promote basal spikelet fertility as well as increase total rachis node number (Fig. 3F). A role for Q’ in delaying conversion of the inflorescence to spikelet meristem is consistent with the recently proposed role of miR172 and AP2 genes in regulating panicle development in rice (Wang et al., 2015).

Wheat spikelets are comprised of two basal glumes (always sterile) followed by an indeterminate number of florets (Fig. 4A). Q’ plants deviated from this fundamental pattern with spikelets often possessing floret structures in place of glumes (Fig. 4B-C, S5). In the basal and apical portion of the spike, glumes were often replaced either by rudimentary florets with only a lemma and palea, or, with increasing frequency towards the terminal spikelet, complete fertile florets (Fig. 4B, C). Florets
occupying typical positions in Q’ formed normally. SEM analysis revealed floret organs forming early in spikelet development adaxial to glume-lemma organs in Q’ (Fig. S5) with no additional lemma-like organ visible. These ectopic florets contributed to an increase in visible florets per spikelet along the spike of Q’ plants (Fig. 4D). Spikelets in the central portion of Q’ spikes were less likely to form florets (partial or complete) in place of glumes (Fig. 4C). In Q’ plants there was a tendency for the glume-like structures of the spikelet to be elongated and produce lemma-like awns, with awn length increasing along the spike from the base to the terminal spikelet (Fig. S6, S7) independent of whether floret structures were visible. Similar phenotypes were observed in MIM172 plants with increased Q expression (Debernardi et al., 2017 companion paper). The simplest interpretation of these Q’ phenotypes is a replacement of glumes with partially or fully developed florets, including awned lemmas. This in turn suggests that increased Q activity promotes ectopic floret formation during spikelet development, and, remarkably, can alter one of the defining features of the grasses, two sterile glumes at the base of each spikelet.

Another typical characteristic of wheat spikes is that a single spikelet is usually present at each rachis node. However, in some genetic backgrounds or under appropriate environmental conditions two spikelets can form at a single node to form a ‘paired spikelet’, which may or may not be the equivalent of a ‘spikelet pair’ in plants such as corn (Boden et al., 2015) (and references therein). While absent in Q and Q’-Rev1 plants, Q’ spikes contained paired spikelets with their frequency peaking around the central rachis nodes of the spike (Fig. S8, S9).

While increased Q activity has multiple effects on spike, spikelet and floret development, the loss-of-function Q’-Rev1 allele also reveals that Q possesses broader, previously unidentified roles (see also Debernardi et al., 2017 companion paper). Unlike Q and Q’, Q’-Rev1 plants intermittently produced spikelets with elongated rachilla internodes and many florets (also known as ‘sham ramification’) (Fig. 4D, E). This trait was more severe in tillers (data not shown) than in the main spike, but the ‘extra’ florets in these spikelets did not produce grains. The sham ramification trait has been mapped to chromosome 5AL in a similar position to Q and has been shown to be repressed by the presence of the D genome in some backgrounds (Alieva and Aminov, 2013, Amagai et al., 2015). In the absence of a D genome, sham ramification and extra florets were observed in tetraploid wheat lines with loss-of-function alleles of Q and in lines overexpressing miR172 (Debernardi et al., 2017 companion paper). Debernardi et al., also report extra sterile glumes in place of florets associated with Q loss-of-function, although we did not observe these traits in our Q’-Rev1 line. Our observations suggest that Q activity must be tightly regulated as both increases (ectopic florets in place of glumes) or decreases (sham ramification) in expression can lead to increases in floret number, similar to reports in maize AP2 mutants (Chuck et al., 2007, Chuck et al., 2008).
Many of the inflorescence architecture defects of $Q'$ are confined to, or more severe in, certain regions of the spike. Most notably, spike compaction (Fig. S10), replacement of glumes with florets and increased awn length all become more severe in nodes closer to the terminal spikelet (Fig. 4, S6, S7). $Q$ expression exhibited temporal and spatial variation during spike development (Fig. S11), with $Q'$ typically more highly expressed than $Q$, and a somewhat reciprocal relationship between $Q$ and miR172 expression, consistent with the results of Debernardi et al., 2017 (companion paper). Increased expression of $Q$ in the peduncle internode and severe reduction in the size of this internode suggests that targeted degradation of $Q$ by miR172 is broadly required to ensure the correct elongation of internodes (stem, rachis and rachilla) in the wheat plant, demonstrating that $Q$ plays an important role throughout wheat reproductive development.

In summary, using mutagenesis and a candidate gene approach we have generated a series of gain- and loss-of-function $Q$ alleles which have allowed us to identify previously unknown aspects of $Q$ gene function in wheat reproductive development. The presumed miRNA mismatches in the gain-of-function mutants we have isolated confirm that the common miRNA regulation of $AP2$ genes also extends to wheat and that correct regulation of $Q$ expression is required for normal formation of the wheat spike and spikelets.
Materials and methods

The spring habit, bread wheat cultivar, Sunstate (SS) was mutagenized using sodium azide as described by (Chandler and Harding, 2013). Sunstate contains the domesticated Q allele. The same mutagenesis was performed on $Q'$ grain when generating revertant alleles. Further information regarding additional Q alleles, threshing and growth conditions can be found in supplementary information.

Developing inflorescence tissue was harvested for qRT-PCR at terminal spikelet stage. 5 developing inflorescences were harvested per biological replicate. Peduncle internode tissue was harvested when the peduncle internode of lines containing the Q domestication allele were 1cm long. A single peduncle internode was harvested for each biological replicate. Sample preparation and qPCR protocol can be found in supplementary information.

For 5' RACE mRNA was purified from the same inflorescence RNA samples used for the initial qRT-PCR analysis of Q in SS-like and $Q'$ plants (Fig 2). A Gene-racer kit (Invitrogen) was used, except the decapping protocol was not carried out, and the adapter was ligated directly to mRNA. Amplification of cleaved and ligated Q transcript was performed using gene specific and Gene-racer adapter specific primers. Amplicon of the expected size was ligated into pGEM®-T easy before transformation, selection and sequencing of individual clones to determine cleavage location and frequency. See supplementary information for more details.

All statistical analysis was performed using two-tailed Students t-test to compare means as indicated in results figure legends. n and P values are stated in results figure legends.
Acknowledgements

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Competing interests

The authors declare no competing interests.

Author contribution

Julian Greenwood performed most of the experiments and led the writing of the manuscript, E. Jean Finnegan helped perform some experiments, Nobuyoshi Watanabe provided genetic material, Ben Trevaskis contributed to experimental design, Steve M. Swain contributed to generation of the mutant population, the experimental design and preparation of the manuscript. All authors made comments on the manuscript.

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Data availability

miR172 sequence shown in this study is of Ta-miR172a obtained from (Yao et al., 2007). Although other isoforms have been reported and may contribute to regulation of Q, Ta-miR172a was used as a reference sequence for alignment purposes.

Q sequence available through GenBank accession: AY702956.1
References


**Figure 1. Identification and characterisation of a compact spike mutant.**

**A,** Adult wheat inflorescence (spike) of Sunstate (SS)-like sibling line, heterozygous line and homozygous mutant line. Scale bar, 3cm **B,** SS-like, heterozygous and homozygous mutant plants at maturity. Scale bar, 30cm **C,** Plant height, **D,** Spike length, **E,** Rachis node number (number of nodes along the spike) and **F,** days to anthesis (flowering time) of SS-like, heterozygous plants and compact spike mutant. Data are presented as mean ± s.e.m n = 33 SS-like, n = 35 Heterozygous, n = 22 Mutant, with significant differences in relation to SS-like shown for heterozygous and mutant plants. *P* < 0.05 **P** < 0.01 ***P** < 0.001
Figure 2. The compact spike mutant, Q', contains a novel SNP in the miRNA-binding site which leads to changes in transcriptional regulation. A, Sequence alignment showing the Q miRNA-binding site sequence of SS-like, with domesticated Q allele (Q), the mutant (Q'), and the complementary wheat miRNA Ta-miR172. The miRNA mismatch underlying the domestication Q allele (C-T) is indicated by the ‘T’ in red boxes, and is present in both Q and Q’. The ‘U’s in red boxes in the miR172 sequence represent mismatches with all known Q sequences. The Q’ mutation is indicated by a red box with a double underline. B, Expression of Q in SS-like and mutant inflorescence tissue harvested at the beginning of internode elongation (~10mm inflorescences at terminal spikelet stage). Data are presented as mean ± s.e.m of 4 biological replicates. *** P < 0.001. C, Developing inflorescence of SS-like (left) and
mutant (right) at the early internode elongation stage where mutant phenotypes first become apparent. Scale bar, 2mm. **D**, Cleavage products as determined by sequencing of 5’RACE products using RNA pooled from the biological samples used for expression analysis in **B**. Number of sequenced clones marked in the red box corresponds to predicted location of cleavage. The number of cleavage products detected is out of sequenced clones containing Q transcript sequence only (20 for SS-like and 17 for Q’).
Figure 3. Co-segregation analysis, loss-of-function mutants derived from \( Q' \) and additional \( Q \) miRNA mutants confirm that the novel SNP is causal for the observed phenotypes of \( Q' \) plants. 

A, Adult wheat inflorescences of SS-like sibling (\( Q \)), \( Q' \) and secondary induced mutants \( Q'\text{-Rev2} \) and \( Q'\text{-Rev1} \) plants in the SS background as well as Novosibirskaya 67 (N67) and \( Q' \)-like mutant ANBW5B Dwarf (5B D) in the N67 background, and sibling lines of ANBW5C Tall (5C T) and ANBW5C Dwarf (5C D, \( Q' \)). Scale bar= 10cm. 

B, Co-segregation of \( Q \) and \( Q' \) in the SS background showing frequency of plants grown from heterozygous parents that fell within specific height range (bins covering 4cm). Frequency bars are coloured depending on the genotype of the plants within those height ranges as determined by CAPS marker analysis.
Plants segregated in agreement with a 1:2:1 ratio as determined by a chi squared test (P = 0.281, n=104 progeny). C, Q gene schematic showing exons (black boxes), introns (thin lines), miRNA target site (grey box) and AP2 binding domains (white boxes). The location of Q’ and Q’-like mutations and derived revertant mutations in Q’ (SS) are shown including nucleotide change and predicted translational changes. D, Plant height E, Spike length and F, Rachis node number of Q, Q’ and Q’-Rev1 plants. Data are presented as mean ± s.e.m, n =10. Relative expression of Q transcript in compact mutant lines normalised to their sibling or parent line in G, developing inflorescences (~10mm inflorescences at terminal spikelet stage) and H, elongating peduncle internode tissue. Data are presented as mean ± s.e.m of 3 biological replicates. * P<0.05 ** P < 0.01 *** P < 0.001
Figure 4. Detailed phenotyping of $Q$, $Q'$ and $Q'$-Rev1 plants reveals inflorescence architecture defects. A, SS-like ($Q$) spikelet image and schematic showing typical wheat spikelet structure where no ectopic florets form. B, $Q'$ spikelet image and schematic showing severe ectopic...
floret phenotype where complete florets replace glumes. C, Q’ plants exhibit complete ectopic florets or floret-like structure in place of glumes most frequently in the base and apical portions of the spike. Data are presented as the proportion of ectopic florets at each rachis node position across 10 plants where the total number of possible glumes =2 per rachis node per plant. A, B, C, P= Palea, Le= Lemma, Gl= Glume, Gr= Grain, Gl-Le= Glume-Lemma. D, Line plots showing floret numbers of sequential spikelets from the base (left) to top (right) of spikes where total spike length =1 for Q, Q’ and Q’-Rev1 plants Data are presented for each of 10 individuals per genotype. Terminal spikelet at distance = 1. Plot points mark individual spikelets with colors indicating separate spikes scored. E, Q’-Rev1 spike indicating elongated spikelets (sham ramification) containing many florets (white circles). Scale bar= 10cm.
Supplementary Materials and Methods

Plant material, threshing and growth conditions

Threshing was performed by hand and by the same person to gauge the threshability of mutant wheat lines. In reference to the loss-of-function mutant Q'-Rev1 threshing required the identification of individual grain for removal by splitting apart florets and spikelets whereas in Q’ and SS-like spikes, grain could easily be separated by rolling the spike back and forth between clapped palms.

The compact mutant near isogenic lines ANBW5B (5B D in Fig 3) and ANBW5C (5C D in Figure 3) were isolated and mapped by (Kosuge et al., 2011). The lines were established by backcrossing the mutants Cp-M808 (ANBW5B) and MCK 2617 (ANBW5C) with Novosibirskaya 67 (N67) for six generations. N67 contains the typical domesticated Q allele. The line 5C T included in Figure 3 represents an N67-like segregant from a line heterozygous for the ANBW5C mutation after backcrossing, however by this round of backcrossing N67 and N67-like segregants are effectively genetically identical.

Screening of mutant lines and phenotyping for co-segregation analysis was performed in glasshouse conditions with temperature controlled to ~22°C (Day) and ~16°C (Night) over Spring and Summer. All other phenotyping and tissue extraction for RNA was from plants grown in cabinets (Conviron PGC20) under 16h light (Measured at 420µM m⁻¹ s⁻¹ 50cm below light source) (22°C) and 8h dark (16°C).

Modified 5’ RACE

mRNA was purified from the same inflorescence RNA samples used for the initial qRT-PCR analysis of Q in SS-like and Q’ plants (Fig 2). A Gene-racer kit (Invitrogen) was used for 5’-RACE, except the decapping protocol was not carried out, and the adapter was ligated directly to mRNA. Amplification of cleaved and ligated Q transcript was performed using gene specific and Gene-racer specific primers. Secondary nested PCR was performed to ensure amplification of gene specific products. Amplification products were gel purified and ligated into pGEM®-T easy before being transformed into E.coli XL-Blue cells and selected on ampicillin plates containing 100 µL of 100mM IPTG and 20 µL of 50 mg/ml X-Gal. Individual colonies were selected and grown overnight in LB with 50 µg/ml ampicillin and purified using Qiagen mini-preps. In total, 96 colonies were selected for sequencing of Q’ (n=48) and SS-like (n=48) amplification product. Only sequenced clones containing correct Q transcript sequence were included with the proportion of cleavage within the miR172-binding site presented. Gene specific primer sequences are listed in Supplementary Table 1.
qRT-PCR

All tissue was ground to fine powder in 1.5ml snap lock tubes containing two ball bearings using a mixermill. RNA extraction and DNASE treatment was performed using the Maxwell® RSC Plant RNA Kit and Maxwell® RSC Instrument following the advised protocol. cDNA synthesis was performed using Maxima H-minus reverse transcriptase from invitrogen with oligo dT to prime mRNA using 5μg total RNA. cDNA was diluted 30 fold and 5μl of diluted cDNA was used in each qPCR reaction. qPCR was performed using SYBR Green on a Roche LightCycler®480II using RP15 as an endogenous control. Primers are shown in Supplementary Table S1.

Routine testing of cDNA samples was performed by serial dilution and calculation of R² from the standard curve. Analysis was performed using Roche LightCycler®480II and associated software. For all cDNA samples presented, 5 log serial dilutions were performed and R² values ≥0.982 were determined before qPCR analysis. Primer efficiency was calculated from the exponential phase of amplification in each reaction sample in each qRT-PCR run. qRT-PCR Primer pairs used in this study routinely achieved amplification efficiencies ≥90%.

Scanning Electron Microscopy (SEM)

Developing inflorescence samples for SEM were prepared by immediate transfer to 100% ethanol after harvest. Ethanol was replaced twice daily until samples appeared completely white (2-3 days). Sample preparation adapted from (Talbot and White, 2013). Samples were then put through a critical point drying process in a tousimis autosamdri®-815 critical point drying device with purge timer set to 4. After critical point drying, samples were mounted on specimen stubs using adhesive carbon discs. Samples were imaged with a Zeiss Evo LS15 scanning electron microscope using backscattered electron detector (Gain= High), at 20KV and extended pressure setting (10pa).
### Supplementary Table S1. Primers used in this study

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miR172 Reverse transcription, expression, control primers associated methods used in this study can be found in companion paper Debernardi et al., 2017.
Supplementary Figures

Supplementary Figure 1 Internode elongation phenotype of $Q'$. Total height of $Q'$ mutant and its sibling line containing the normal $Q$ domestication allele with total height being shown as stacked histogram of average spike, peduncle internode, peduncle minus 1 and remaining basal internode lengths. n= 12 plants. The number of basal peduncle internodes varied between two and three visible internodes amongst measured plants. The length of peduncle and peduncle internode were measured individually as differences in the length of these internodes, as well as the spike, accounted for most of the height reduction in $Q'$ plants.

Supplementary Figure 2 miRNA-binding site alignments of pre-domesticated $q$, domesticated $Q$, gain-of-function alleles $Q'$ and $Q'$-like. miRNA-binding site alignments and complementary $miRNA172$ with mismatches shown in red and induced mismatches shown by red double underline.
Supplementary Figure 3 The loss-of-function Q’-Rev1 is difficult to thresh A mechanical thresh test was performed by applying downward pressure to whole spikes with a hand threshing tool before sliding the tool horizontally. SS-like plants containing the domestication allele Q had spikes that were easy to thresh and Q’ plants had spikes that were very easy to thresh with all grain separating from the head leaving an intact rachis. In contrast, Q’-Rev1 plants had spikes that were firm and required more pressure to thresh with some grain not separating from the spikelets. In addition, the rachis of Q’-Rev1 spikes had a greater tendency to break into individual nodes/spikelets. In each image the threshed rachis and intact spikelets have been arranged on the left hand side of the image. In Q and Q’ plants, no grain remained bound in spikelets unlike Q’-Rev1.

Supplementary Figure 4 The Q’ mutant reduces the number of basal rudimentary (sterile) spikelets and produces fertile spikelets at basal rachis nodes that are unfilled in Q and the loss-of-function mutant Q’-Rev1. A, Basal portion of Q, Q’ and Q’-Rev1 spikes. Rachis nodes with rudimentary spikelets (those lacking developed florets) are marked *. Scale bar= 1cm. B, The average number of infertile spikelets (those that produced no grain) and the number of infertile spikelets which appeared to be rudimentary (without florets) in the basal portion of the main spike of Q, Q’ and Q’-Rev1 plants. n= 10. ** P < 0.01
Supplementary Figure 5 Ectopic florets are produced in Q′ spikelets. SEMs of SS-like (Left: A, C, E) and Q′ (Right: B, D, F) spikes. Outer-most bracts have been removed from selected spikelets in C, D, E, F to reveal structures beneath. In the case of Q′ ectopic florets (Fl*) are visible beneath the outermost bracts of apical B, and dissected D, F, spikelets, while the outer-most bracts (glumes) of SS-like plants A, C, E feature typical sterile glumes. Scale bars= 200µm
Supplementary Figure 6 Glumes of Q’ mutant appear lemma-like with awns present in increasing length toward the apical portion of the spike. Glumes of Q, Q’ and Q’-Rev1 plants removed from one side of a single spike and placed in order to reflect their position on an intact and upright spike. Note that glumes of Q’, or the outermost positions on spikelets which should be occupied by glumes, are only similar to the glumes of Q when comparing basal spikelets. Scale bar= 1cm

Supplementary Figure 7 Outermost bracts of Q’ spikelets exhibit elongation of tips as in lemmas. SEMs of spikes shown are at comparable stages of inflorescence development when the tips of lemmas (awns) are beginning to elongate in both SS-like (Q) and Q’ plants. The outermost bracts of Q’ spikelets B, feature elongating tips much like a lemma at this stage whereas the outermost bracts of Q spikelets A, do not elongate and maintain short tips until maturity. See also S Fig 5. Gl= glume, Le= Lemma and Gl-Le= Glume-Lemma. Scale bars= 200µm
Supplementary Figure 8 Pairs of spikelets form at the central rachis nodes of Q' spikes. SEMs of dissected SS-like (Q) A, and Q' spikes B, C, D, E, to reveal the base of selected spikelets where spikelet pairs form, the spikelet beneath has been removed. The base of the SS-like spikelet shown in A, represents a typical single spikelet formation. The base of Q’ spikelets in B and D show the formation of additional, or paired spikelet at the base of other spikelets. Scale bars= 200µm

Supplementary Figure 9 Proportion of paired spikelets in Q’ plants forming at each rachis node. Paired spikelets at specific rachis node positions in Q’ plants are shown as proportion of all spikelets scored at that rachis node position in main spikes of Q’ plants. n= 10. Complete paired spikelets contained at least one macroscopic floret.
Supplementary Figure 10 Rachis internode elongation is reduced in plants containing the Q’ with the most severe reduction in internode length occurring at the top of the spike. Rachis internode length profiles of SS-like (Q Q), heterozygous (Q Q’) and homozygous (Q’ Q’ ) mutants where 1 equals the most basal rachis internode of the main spike. n=12. As rachis internode number varies between individuals, each individual has been plotted and represented by a different color.
Supplementary Figure 11 Spatial and temporal expression of Q and miR172. Images along the bottom of this figure correspond to the developmental stage at which spike sections were harvested. Expression analysis is shown relative to control genes for both Q and miR172. Data are presented as mean ± s.e.m of 4 biological replicates in each section, at each timepoint.

Supplementary References


