A stepwise route to domesticate rice by controlling seed shattering and panicle shape

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Abstract

Rice (*Oryza sativa* L.) is consumed by more than half of the world's population, but despite its global importance the mechanisms of domestication remain unclear. During domestication, wild rice (*O. rufipogon* Griff.) was transformed by acquiring non-seed-shattering behaviour, an important genetic change that allowed humans to increase grain yield. However, we show previously identified loci, *sh4* and *qSH3*, are individually insufficient to explain loss of seed shattering nor increases in harvest yield in wild rice. We identify the complementary interaction of key mutations for abscission layer interruption and panicle architecture that were causal in the early domestication of Asian rice. An interruption of abscission layer formation requires both *sh4* and *qSH3*, which presents an apparent barrier to selection of shattering loss. We identified the causal single nucleotide polymorphism at *qSH3* within a seed-shattering gene *OsSh1* conserved in *indica* and *japonica* subspecies, but absent in the *circum*-aus group of rice. We demonstrate through harvest experiments that seed-shattering alone does not significantly impact yield. Instead, we observed yield increases under a *SPR3*-controlled closed panicle formation, which is augmented by the integration of *sh4* and *qSH3* alleles causing a slight inhibition of abscission layer. Complementary manipulation of seed shattering and panicle shape result in a panicle structure that is mechanically stable. We propose a stepwise route in the earliest phase of rice domestication in which selection for visible *SPR3*-controlled closed panicle morphology was instrumental in the sequential recruitment of *sh4* and *qSH3* and leading to loss of shattering.

Significance Statement

Rice is one of the most important crops worldwide. Loss of seed shattering in domesticated rice, previously attributed to single mutations such as in *sh4*, is considered the principal genetic change which resulted in yield increases. However, we show that *sh4* is insufficient on its own to cause abscission layer disruption and other genes, such as *qSH3* are required, making mechanisms for the initial selection of non-shattering unclear. We show that shattering loss in wild rice genetic backgrounds does not increase yields. We identify an interaction in which a second trait, closed panicle formation controlled by *SPR3*, both increases yield and facilitates recruitment of *sh4* and *qSH3* which synergistically augment yield, leading to a stepwise route for rice domestication.

Main Text

Introduction

Selection of naturally occurring variations in wild plants that provided useful agronomic traits was an essential step for crop domestication. These traits are often related to yield, such as seed number, seed size and a loss of seed dispersal, as well as ease of cultivation, including plant architecture, seed dormancy, and photoperiod sensitivity. Plants with these traits provided the necessary impetus for humans to shift from hunting and gathering to cultivating. The visible morphological changes were key for triggering domestication in many crop species. Among several domestication-related traits, the suppression of seed shattering is considered the most important genetic change that allowed humans to increase grain yield and differentiated domesticated from wild plants (1, 2).

Rice (*Oryza sativa* L.) is consumed by almost half of the world's population and is particularly important in Asia. During its domestication, wild rice (*O. rufipogon* Griff.) was transformed by acquiring non-seed-shattering behaviour. Investigations on prehistoric rice spikelet bases from archaeological sites demonstrate that suppression of seed shattering leaves a phenotypic trace that can be observed through the examination of the abscission layer (3). Archaeological rice spikelet bases with rough and deeply torn scars in the spikelet base (rachilla) indicate that humans had to actively detach the spikelets from the pedicels by threshing. More than a decade ago, two loci, *qSH1* and *sh4*, were identified as major loci that influence the reduction in seed shattering based on quantitative trait locus (QTL) analyses (4, 5). Since *sh4* and *qSH1* displayed large phenotypic variances, they were recognised as crucial to rice domestication.

To better understand how *qSH1* and *sh4* were involved in the loss of seed shattering in wild rice, we developed introgression lines (ILs) named IL(*qSH1*-N) and IL(*sh4*-N), which contain small chromosomal segments from *O. sativa* Nipponbare at the *qSH1* and *sh4* loci, respectively, in the genetic background of wild rice *O. rufipogon* W630 (6). Complete seed shattering was still observed in these lines, indicating that neither of the single mutations at *qSH1* and *sh4* could account for non-shattering during rice domestication. As the *sh4* mutation is commonly observed

in cultivated rice, and the *qSH1* mutation is only found in some *japonica* cultivars (4), additional mutation(s) together with *sh4* must have played a role in reducing seed shattering during the early stages of rice domestication.

In this study, we conducted genetic analysis of the *qSH3*, an additional locus involved in the loss of seed shattering together with *sh4*, and identified the causal mutation selected during rice domestication. The frequency of the *qSH3* alleles in rice cultivars and their effects on the loss of seed shattering were evaluated. In addition, we assessed the interaction between seed shattering and panicle shape, and also investigated their role in seed shattering behaviour based on structural mechanic analysis to better understand the selection process on a loss of seed shattering in rice.

Results and discussion

Identification of the causal mutation at *qSH3*, a locus involved in the loss of seed shattering during rice domestication

To identify additional genomic regions involved in reducing seed shattering, we produced an IL with chromosomal segments of *O. sativa* Nipponbare at both *sh4* and *qSH1* in the genetic background of wild rice, *O. rufipogon* W630 (7). IL(*qSH1*-N+*sh4*-N) was crossed with Nipponbare, and their F_2 population was subjected to QTL analysis to determine the degree of seed shattering, and the locus *qSH3* was identified (7, 8). The effect of *qSH3* was confirmed in the wild genetic background as IL(*qSH1*-N+*sh4*-N+*qSH3*-N) showed reduced seed-shattering behaviour compared with IL(*qSH1*-N+*sh4*-N) (7). To identify the gene for *qSH3*, we performed high-resolution linkage analysis using a mapping population from a cross between IL(*qSH1*-N+*sh4*-N) and Nipponbare (9) (Fig. S1). The genomic location of *qSH3* was delimited by the two recombinants to a 1,228 bp region that harboured a part of a previously known seed-shattering gene, *OsSh1* (*Os03g0650000*) (Figs. S2 and S3). This gene was identified as a homolog of *Sh1*, which controls abscission layer formation in sorghum (10). The function of *OsSh1* in seed shattering was studied using artificially induced rice materials showing that the null mutations caused a complete loss of seed shattering

(10, 11), but the causal mutation selected in rice domestication was not determined. A sequence survey in the 1,228 bp region of 5′ UTR, exon 1, and intron 1 of *OsSh1* identified seven polymorphisms between Nipponbare and W630 (Fig. 1A and Table S1). Among them, only a single nucleotide polymorphism (SNP), namely SNP-70, was located in the coding region (exon1) of *OsSh1*, causing an amino acid substitution from leucine in W630 to phenylalanine in Nipponbare (Fig. S4). To test whether SNP-70 is associated with the degree of seed shattering, we performed a transformation test in the Nipponbare genetic background of two constructs that differed only at the SNP-70 position. In the preliminary experiment, we observed that IL(*qSH3*-W), carrying the W630 allele at *qSH3* in Nipponbare genetic background (Fig. S5), showed a non-seed-shattering behaviour similar to Nipponbare, whereas IL(*qSH1*-W+*qSH3*-W) displayed a significantly higher seed-shattering degree than the IL(*qSH1*-W) (Fig. 1B and Fig. S6). These findings indicate that the seed-shattering effect of *qSH3* was considerably enhanced by a functional allele at *qSH1*. Therefore, we introduced two types of constructs carrying the *qSH3* cDNA sequence of W630 ($qSH3^{W630}$) and Nipponbare ($qSH3^{Npb}$) into IL(*qSH1*-W) (Fig. 1C). As a result, transformants with $qSH3^{W630}$ showed enhanced seed shattering, compared with the control IL(*qSH1*-W), whereas those with $qSH3^{Npb}$ did not (Fig. 1D), confirming that SNP-70 is the causal mutation for reduced seed shattering.

Causal mutation at *qSH3* is conserved in both *japonica* and *indica* but not in *circum*-aus rice cultivars

Next, we analysed the distribution of SNP-70 at *qSH3* in cultivated rice. First, genotyping at the causal SNPs of *sh4*, *qSH1*, and *qSH3* was conducted for the three rice cultivars, Nipponbare, IR36, and Kasalath, belonging to *japonica*, *indica*, and *circum*-aus, respectively. All cultivars possessed the causal mutation at *sh4* and only Nipponbare had the causal mutation at *qSH1*. As for *qSH3*, Nipponbare and IR36 carried the causal mutation, but it was absent in Kasalath (Fig. 2A). We further found that the Kasalath haplotype is similar to that of W630 around the *qSH3* region (Table S1). Using the diverse varieties of the World Rice Core Collection (WRC) (12), we found

that 14 lines, all belonging to *circum*-aus, carried the functional allele at *qSH3* (Table S2 and Figs. S7 and S8). Furthermore, the Rice 3K genome project data (13) clearly showed that both *indica* and *japonica* carried the causal mutation, but almost 90% of *circum*-aus rice carried a functional allele at *qSH3* (Fig. 2B). To further understand the footprint of *qSH3* selection, we analysed nucleotide diversity across the *qSH3* genomic region. Using sequences of the Rice 3K genome collection (13), we detected a selective sweep at *qSH3* in both *indica* and *japonica*, but not in the *circum*-aus linage (Fig. 2C, Figs. S9 and S10). These results suggest that *circum*-aus may have experienced a separate trajectory to evolve reduced seed shattering (14). Thus, the reduction in seed shattering is dependent on lineage-specific variations in the subspecies, which is key to understanding the processes of rice domestication.

Role of *qSH3* causal mutation in an initial loss of seed shattering

We next aimed to understand how the causal SNP at *qSH3* contributed to initial rice domestication by reducing seed shattering in *japonica* and *indica*. Since the *sh4* mutation is conserved in all cultivated rice, we produced IL(*sh4*-N) and IL(*qSH3*-N) in the genetic background of wild rice *O. rufipogon* W630. Complete formation of the abscission layer similar to W630 was observed in both ILs (Fig. 3), suggesting that the single mutation at each locus was insufficient for phenotypic change in the abscission layer formation. However, a slight inhibition of the abscission layer around vascular bundles was observed in IL(*sh4*-N+*qSH3*-N) (Fig. 3). A slight abscission layer inhibition was also observed in several wild rice accessions of *O. rufipogon* carrying mutations at both *sh4* and *qSH3* (Figs. S11 and S12, and Table S3), although they may have gained these domestic-type alleles through introgressions from cultivated rice (15). Even if the mutations in the two loci displayed phenotypic difference in the abscission layer formation adequate to reduce shattering under a greenhouse conditions without wind, but their effect on seed shattering was much less than expected under field conditions.

Seed-shattering behaviour associated with a slight inhibition of abscission layer was mitigated by a closed panicle formation

Previously, we reported that a closed panicle trait controlled by *OsLG1* had a major effect in rice domestication by facilitating grain harvest (1). A closed panicle reduces seed shedding by retaining seeds that get stuck by long awns found in the lower sections of the panicle. Interestingly, the *SPR3* (a locus regulating *OsLG1*) region was under strong selection in *indica*, *japonica*, and *circum*-aus (Fig. S13), suggesting that the closed panicle trait was selected in the early phase of rice domestication and so might have been associated with seed-shattering changes, although this trait, unlike seed shattering, is not visible archaeologically. Therefore, we generated seven wild ILs with different combinations of the three loci (*sh4*, *qSH3*, and *SPR3*) (Fig. S14) and compared their morphology together with the wild rice *O. rufipogon* W630 (Fig. S15). The heading date of all ILs was similar to those of W630 (Fig. S16), but with distinct abscission layer formation (Fig. 3 and Fig. S17) and panicle structure (Figs. 4A and 4B). Small BTS values were observed only for IL(*sh4*-N+*qSH3*-N) and IL(*sh4*-N+*qSH3*-N+*SPR3*-N), while the rest were close to zero as wild rice (Fig. 4C). We were interested in assessing the differences in yields brought about by the causal mutations and which combination of alleles would have provided humans in prehistory with higher yields, including whether a closed panicle conferred additional value to humans when gathering wild rice. We therefore subjected the seven ILs and W630 to a seed-gathering experiment in the field (Fig. S18, Movies S1 and S2). The seed-gathering rate from IL(*sh4*-N) and IL(*qSH3*-N), and IL(*sh4*-N+*qSH3*-N) was not significantly different from that of W630 (Fig. 4D and Table S4). However, the three ILs with a closed panicle structure, IL(*SPR3*-N), IL(*sh4*-N+*SPR3*-N), and IL(*qSH3*-N+*SPR3*-N), presented a slightly increased yield. In contrast, IL(*sh4*-N+*qSH3*-N+*SPR3*-N), with a combination of closed panicle and abscission layer inhibition, showed a significant increase in gathering rate compared with W630 (Fig. 4D and Table S4). As closed panicles would be easily visible in the field, humans could have targeted this higher yielding rice (Fig. S19).

When plants were sown from harvests, closed panicles would have been selected for, and unintentionally would have selected also for reduced shattering, because of the enhanced

gathering rate that would occur in the presence of the domesticated alleles. As these alleles increased in frequency and became fixed this would have increased yields, which would have encouraged further investment in rice cultivation (16). The selection for closed panicle instigates self-pollination behaviour owing to the long awns, which disturb the free exposure of anthers and stigmas (stigmas and closed panicle) (17). Thus, a closed panicle may also be advantageous in mitigating natural variations in seed-shattering loci by reducing outcrossing. Although awns in modern cultivated rice are undesirable, gathering of rice in the early stages of rice domestication might have benefitted from the presence of awns in plants with closed panicles, as these would have increased yield and self-pollination rates.

Complementary interaction of a slight inhibition of abscission layer and closed panicle formation synergistically contributed to structural stability of panicle to increase yield

To better understand how the closed panicle, caused by *SPR3*, and the inhibition of abscission layer, caused by *sh4* and *qSH3*, contributed to the initial loss of seed shattering, we analysed their roles by performing structural mechanics analysis. The awns of wild rice play a pivotal role in seed dispersal in spreading panicles (Fig. 5A). We measured the lengths and weights of awns and grains in wild rice *O. rufipogon* W630, as well as calculated the sectional force exerted on the spikelet base depending on panicle angles (Fig. 5B). The axial and shear forces were slightly increased in a closed panicle compared to an open panicle (Fig. S20). However, the bending moment, which is the predominant factor affecting seed dispersal in an open panicle, was considerably reduced in a closed panicle (Fig. S20). A slight inhibition of the abscission layer by *sh4* and *qSH3* led to an increase in the length of abscission layer inhibition (Fig. 3). Therefore, we measured the length of the abscission layer and central vascular bundle in *O. rufipogon* W630 by scanning microscopy (Figs. 5C and D). We also calculated the moment of inertia of the area, a property that describes the torque required to break the abscission layer and so disarticulate the grain, for the disrupted abscission layer (Fig. S20). The value increased exponentially with increasing lengths of abscission layer inhibition. A reduction in the bending moment and an

increase in the moment of inertia of the area act synergistically to reduce bending stress (Fig. 4E), contributing to the structural stability of spikelets without shattering. Thus, the interaction between a closed panicle and abscission layer inhibition acted complementarily to increase yield.

Conclusions

In this study, we identified key functional genes that contributed to rice domestication by increasing harvest yields. We identified the causal SNP at *qSH3* involved in reduced seed shattering, whereas the previously proposed *sh4* mutation alone could not trigger the non-shattering morphology in rice (6, 17). We also explained how the early selection of a closed panicle and the resulting mechanics of spikelet retention would have increased yields and facilitated the selection for non-shattering. Our results showed that the initial step of rice domestication is more complex than previously thought. With new information from our studies, a change in the long-held perspective from a single domestication allele model to one where synergistic effects of several domestication genes is needed.

The origin and spread of rice subspecies based on population genetic analysis have been the subject of discussions (14, 18-21). However, most of the studies have been conducted using the genome information of modern cultivars and wild rice, without considering the importance of visible phenotypic changes targeted by humans. Therefore, the lineage-specific variations associated with quantitative traits are a key to further understand the process of rice domestication.

Based on our work, we propose a stepwise route for rice domestication. In wild rice, any of the natural variations in the loci for seed shattering and closed panicle formation alone had little effect on increasing yield (Fig. 4). Their combination, however, established an archaic rice which could have been recognised by ancient gatherers as advantageous to increasing yields. The change in harvesting efficiency along with the use of harvesting tools further promoted the selections of natural variants in domestication-related traits in rice, a crop which now supports billions of people worldwide.

Materials and Methods

Details regarding plant materials, QTL mapping, fine mapping, transformation, population genetic analysis, histological analysis, seed gathering experiment, and structural mechanics analysis are provided in the Supplementary Information.

Figure legends

Fig. 1. Identification of a causal SNP of *qSH3* associated with the degree of seed shattering. (*A*) Fine mapping of the causal mutation at *qSH3* in a 1,228-bp region on chromosome 3. A causal SNP (SNP-70) identified in *OsSh1* (*Os03g0650000*) is shown in bold. (*B*) Comparison of seed-shattering degree by breaking tensile strength (BTS) values in three introgression lines, IL(*qSH3*-W), IL(*qSH1*-W), and IL(*qSH1*-W+*qSH3*-W) in the Nipponbare genetic background. Data are mean ± s.d. of four plants. n.s. and ** indicate not significant and significant at the 1% level based on unpaired Student's t-test, respectively. (*C*) Two types of constructs carrying *qSH3* cDNA sequences of W630 and Nipponbare ($qSH3^{W630}$ and $qSH3^{Npb}$) driven by a 3-kb region of the promoter used for transgenic analysis. (*D*) The BTS values observed for the transformants with $qSH3^{W630}$ and $qSH3^{Npb}$. Black triangles represent the average BTS value of IL(*qSH1*-W).

Fig. 2. Lineage-specific selection at *qSH3* locus in rice. (*A*) Genotyping at *qSH3*, *sh4*, and *qSH1* for *O. rufipogon* W630, *O. sativa japonica* Nipponbare, *indica* IR36, and *circum*-aus Kasalath based on the causal SNPs by dCAPS markers. (*B*) Allele frequency of *qSH3* causal SNP (%) in cultivated rice based on the 3K rice project data. Nipponbare type ('T') and W630 type ('C') are shown in yellow and green, respectively. (*C*) Nucleotide diversity (π) observed for the domesticated rice in the physical position of 25.0-25.3 Mb on chromosome 3. A flanking region of the *qSH3* locus (around 25.2 Mb) was substantially decreased in *japonica* and *indica* cultivars. π was calculated in 10-kb windows using SNPs data of the 3K rice project (13).

Fig. 3. Abscission layer formation partially inhibited by *sh4* and *qSH3* in wild rice.

Abscission layer formation in *O. rufipogon* W630, IL(*sh4*-N), IL(*qSH3*-N), IL(*sh4*-N+*qSH3*-N), and *O. sativa* Nipponbare. Each enlarged section indicated by the dotted square is shown in the lower panel. A black triangle indicates an inhibited area of the abscission layer observed for IL(*sh4*-N+*qSH3*-N). Scale bars = 100 μm.

Fig. 4. Role of the *sh4*, *qSH3*, and *SPR3* mutations on the initial loss of seed shattering in rice domestication.

(*A*, *B*) Panicle shape of the seven ILs at flowering (*A*) and seed-maturation (*B*) stages. Scale bars = 5 cm (*C*) Breaking tensile strength (BTS) values for the seven ILs. BTS values for *O. sativa japonica* Nipponbare and *indica* IR36 are shown as control. n.s. indicates not significant by unpaired Student's t-test. (*D*) Seed-gathering rates (mean ± s.d. of four plot replicates, with nine plants in each plot) for *O. rufipogon* W630 and the seven ILs. Mean values labelled with different letters are significantly different, whereas those with the same letters are not (Tukey's test with arcsine transformed values, $P < 0.05$).

Fig. 5. Structural mechanics analysis of panicle shape and abscission layer inhibition for the initial loss of seed shattering.

(*A*), Panicle shape of *O. rufipogon* W630. Bar = 5cm. (*B*) Schematic representation of awn and grain with panicle angle. θ represents the panicle angle. l_1, l_2, m_1g, and m_2g indicate the length of grain, awn, weight of grain and awn, respectively. (*C*) Detachment of grain and pedicel (left) and scanning electron microscopy (SEM) analysis of the abscission layer of pedicel in *O. rufipogon* W630 (right). AL and VB indicate abscission layer and vascular bundle, respectively. Bars = 1 mm (left) and 100 μm (right). (*D*) Schematic representation of the abscission layer. *D* and *d* indicate the diameter of abscission layer and vascular bundles, respectively. Dotted circle indicates the area of disrupted abscission layer. (*E*) Simulation of the bending stress exerted on the spikelet base depending on panicle shape and abscission layer inhibition, with higher stress experience in open panicle with less inhibited abscission.

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Fig. 1

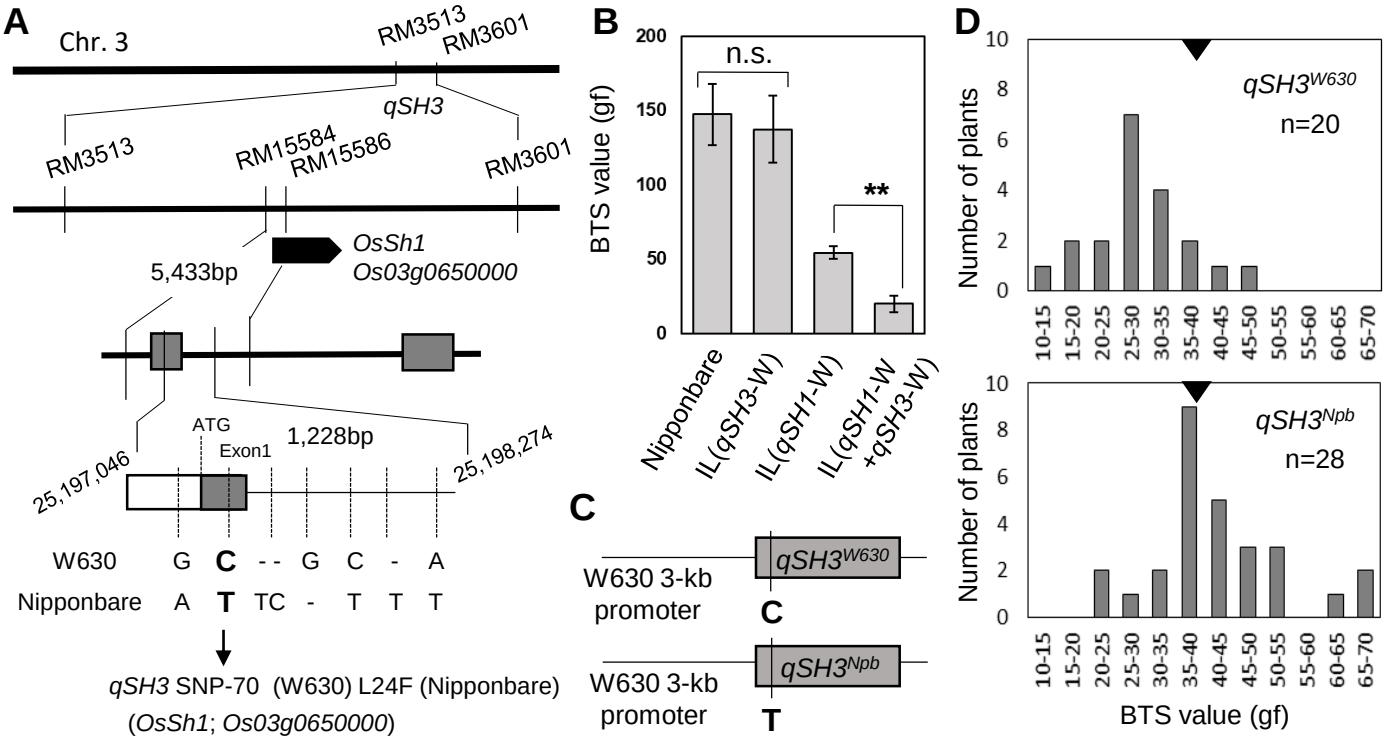

Fig. 1. Identification of a causal SNP of *qSH3* associated with the degree of seed shattering.

(*A*) Fine mapping of the causal mutation at *qSH3* in a 1,228-bp region on chromosome 3. A causal SNP (SNP-70) identified in *OsSh1* (*Os03g0650000*) is shown in bold. (*B*) Comparison of seed-shattering degree by breaking tensile strength (BTS) values in three introgression lines, IL(*qSH3*-W), IL(*qSH1*-W), and IL(*qSH1*-W+*qSH3*-W) in the Nipponbare genetic background. Data are mean ± s.d. of four plants. n.s. and ** indicate not significant and significant at the 1% level based on unpaired Student's t-test, respectively. (*C*) Two types of constructs carrying *qSH3* cDNA sequences of W630 and Nipponbare ($qSH3^{W630}$ and $qSH3^{Npb}$) driven by a 3-kb region of the promoter used for transgenic analysis. (*D*) The BTS values observed for the transformants with $qSH3^{W630}$ and $qSH3^{Npb}$. Black triangles represent the average BTS value of IL(*qSH1*-W).

Fig. 2

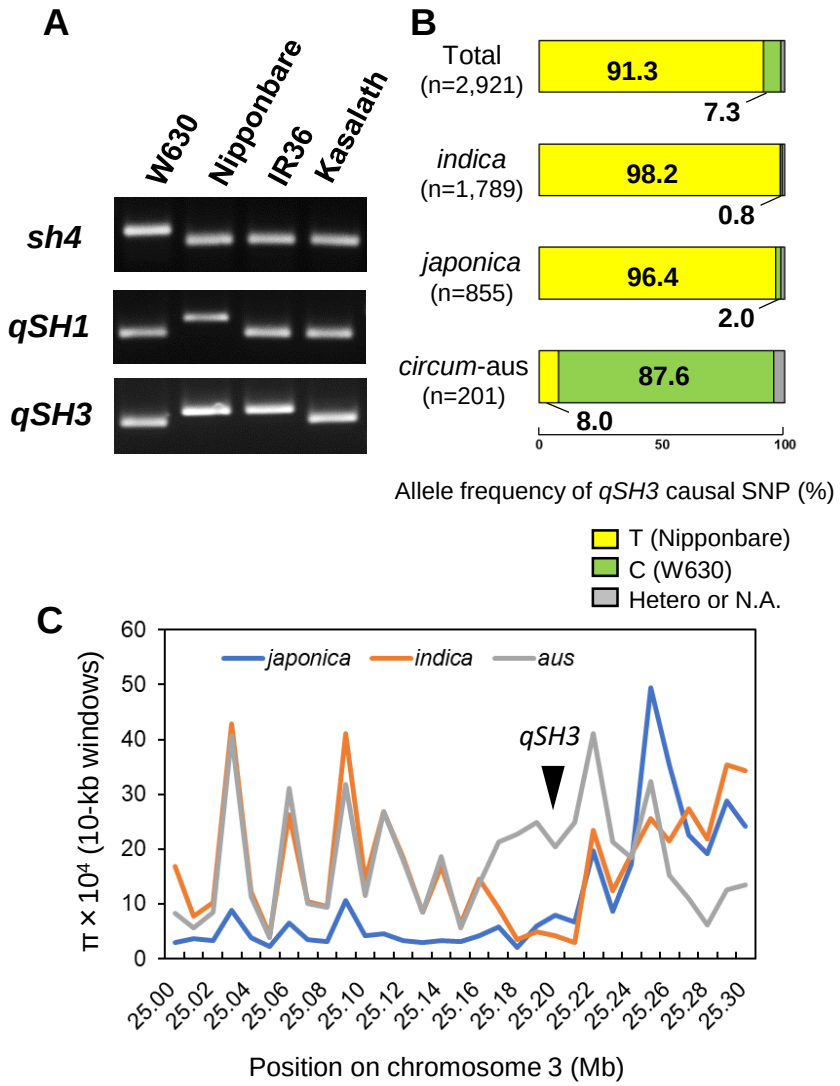

Fig. 2. Lineage-specific selection at *qSH3* locus in rice.
(*A*) Genotyping at *qSH3*, *sh4*, and *qSH1* for *O. rufipogon* W630, *O. sativa japonica* Nipponbare, *indica* IR36, and *circum*-aus 'Kasalath' based on the causal SNPs by dCAPS markers. (*B*) Allele frequency of *qSH3* causal SNP (%) in cultivated rice based on the 3K rice project data. Nipponbare type ('T') and W630 type ('C') are shown in yellow and green, respectively. (*C*) Nucleotide diversity (π) observed for the domesticated rice in the physical position of 25.0-25.3 Mb on chromosome 3. A flanking region of the *qSH3* locus (around 25.2 Mb) was substantially decreased in *japonica* and *indica* cultivars. π was calculated in 10-kb windows using SNPs data of the 3K rice project (13).

Fig. 3

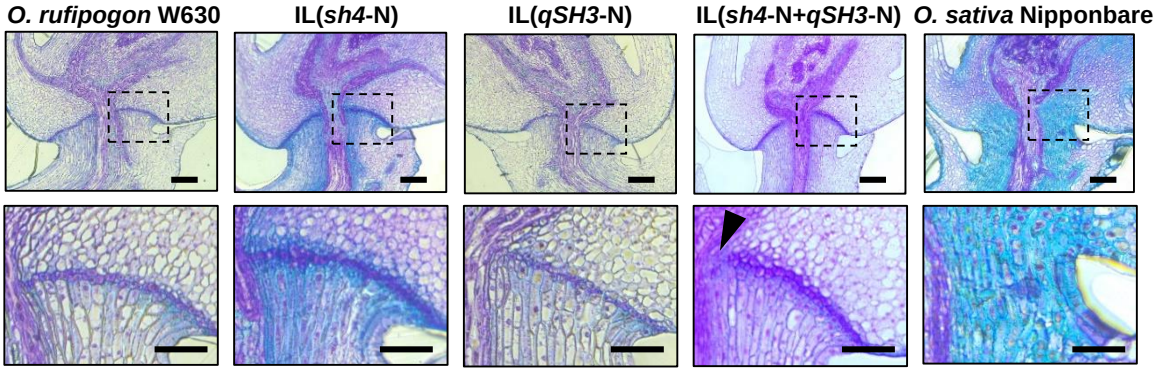

Fig. 3. Abscission layer formation partially inhibited by *sh4* and *qSH3* in wild rice.

Abscission layer formation in *O. rufipogon* W630, IL(*sh4*-N), IL(*qSH3*-N), IL(*sh4*-N+*qSH3*-N), and *O. sativa* Nipponbare. Each enlarged section indicated by the dotted square is shown in the lower panel. A black triangle indicates an inhibited area of the abscission layer observed for IL(*sh4*-N+*qSH3*-N). Scale bars = 100 μm.

Fig. 4

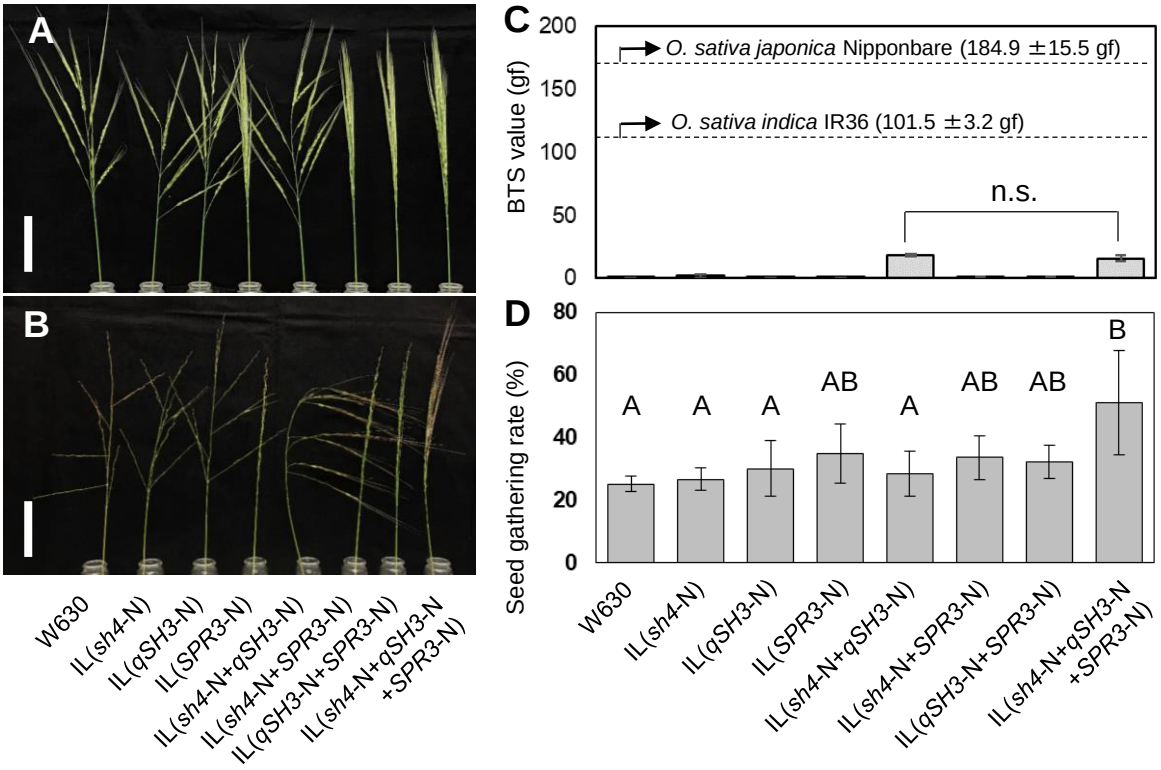

Fig. 4. Role of the *sh4*, *qSH3*, and *SPR3* mutations on the initial loss of seed shattering in rice domestication.

(*A*,*B*) Panicle shape of the seven ILs at flowering (*A*) and seed-maturation (*B*) stages. Scale bars = 5 cm (*C*) Breaking tensile strength (BTS) values for the seven ILs. BTS values for *O. sativa japonica* Nipponbare and *indica* IR36 are shown as control. n.s. indicates not significant by unpaired Student's t-test. (*D*) Seed-gathering rates (mean ± s.d. of four plot replicates, with nine plants in each plot) for *O. rufipogon* W630 and the seven ILs. Mean values labelled with different letters are significantly different, whereas those with the same letters are not (Tukey's test with arcsine transformed values, $P < 0.05$).

1

Fig. 5

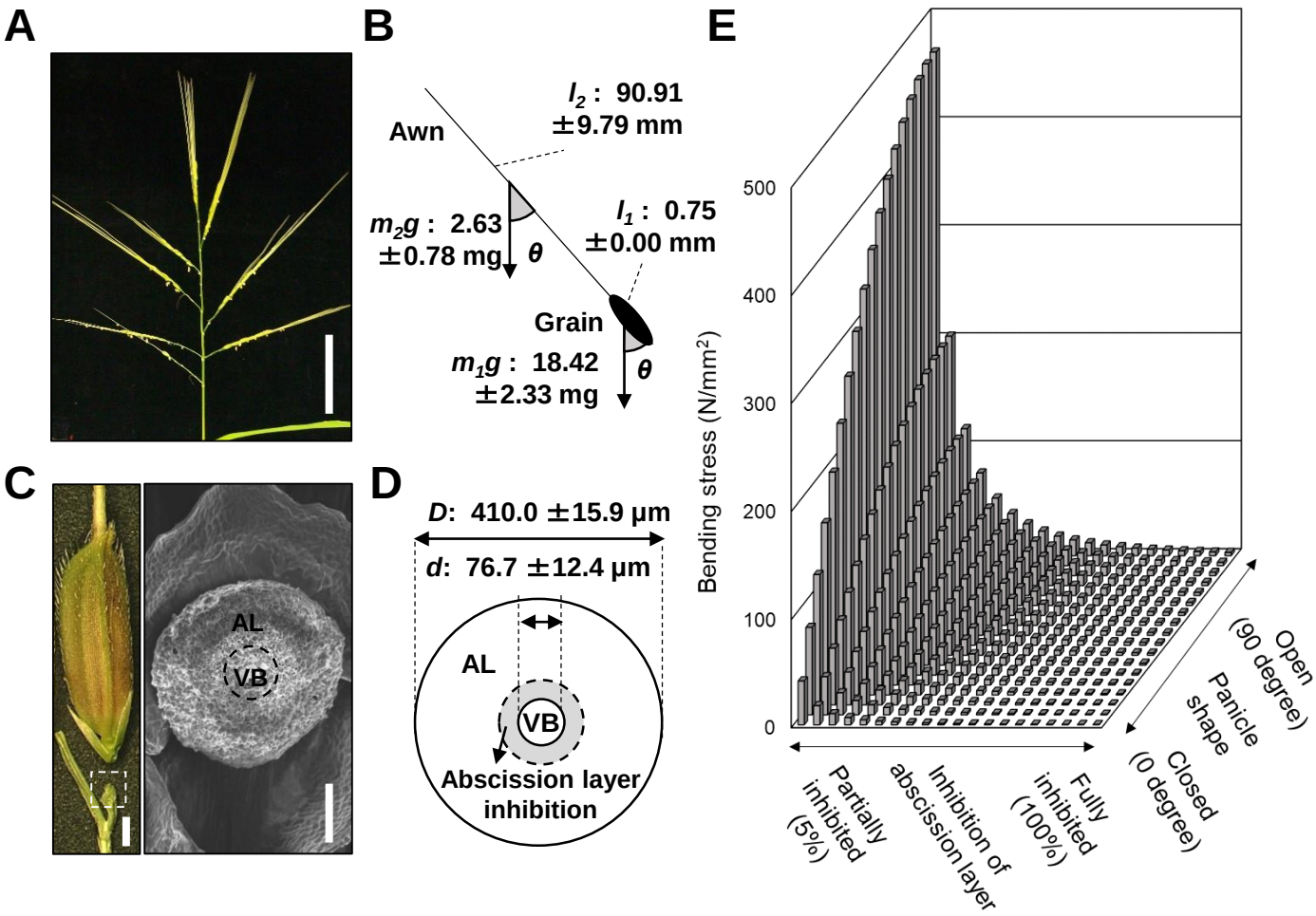

Fig. 5. Structural mechanics analysis of panicle shape and abscission layer inhibition for the initial loss of seed shattering.
(*A*), Panicle shape of *O. rufipogon* W630. Bar = 5cm. (*B*) Schematic representation of awn and grain with panicle angle. *θ* represents the panicle angle. l_1, l_2, m_1g, and m_2g indicate the length of grain, awn, weight of grain and awn, respectively. (*C*) Detachment of grain and pedicel (left) and scanning electron microscopy (SEM) analysis of the abscission layer of pedicel in *O. rufipogon* W630 (right). AL and VB indicate abscission layer and vascular bundle, respectively. Bars = 1 mm (left) and 100 μm (right). (*D*) Schematic representation of the abscission layer. *D* and *d* indicate the diameter of abscission layer and vascular bundles, respectively. Dotted circle indicates the area of disrupted abscission layer. (*E*) Simulation of the bending stress exerted on the spikelet base depending on panicle shape and abscission layer inhibition, with higher stress experience in open panicle with less inhibited abscission.