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

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Asian rice (*Oryza sativa* L.) is consumed by more than half of the world's population. Despite its global importance, the process of early rice domestication remains unclear. During domestication, wild rice (*Oryza rufipogon* Griff.) acquired non-seed-shattering behavior, allowing humans to increase grain yield. Previous studies argued that a reduction in seed shattering triggered by the *sh4* mutation led to increased yield during rice domestication, but our experiments using wild introgression lines show that the domesticated *sh4* allele alone is insufficient for shattering loss in *O. rufipogon*. The interruption of abscission layer formation requires both *sh4* and *qSH3* mutations, demonstrating that the selection of shattering loss in wild rice was not as simple as previously suggested. Here we identified a causal single-nucleotide polymorphism at *qSH3* within the seed-shattering gene *OsSh1*, which is conserved in *indica* and *japonica* subspecies but absent in the *circum*-aus group of rice. Through harvest experiments, we further demonstrated that seed shattering alone did not significantly impact yield; rather, yield increases were observed with closed panicle formation controlled by *SPR3* and further augmented by nonshattering, conferred by integration of *sh4* and *qSH3* alleles. Complementary manipulation of panicle shape and seed shattering results in a mechanically stable panicle structure. We propose a stepwise route for the earliest phase of rice domestication, wherein selection of visible *SPR3*-controlled closed panicle morphology was instrumental in the sequential recruitment of *sh4* and *qSH3*, which together led to the loss of shattering.

Oryza sativa | *Oryza rufipogon* | domestication | seed shattering | closed panicle

Significance

Rice is one of the most important crops worldwide. Loss of seed shattering in domesticated rice, previously attributed to single-gene mutations such as *sh4*, is reported to be the essential genetic change resulting in yield increases during domestication. However, we show that *sh4* alone is insufficient, and other genes, such as *qSH3*, are required to cause abscission layer disruption. The evolution of non-seed-shattering behavior therefore required multiple mutations. Furthermore, shattering loss in the genetic background of wild rice does not increase yield. We demonstrate that closed panicle formation controlled by *SPR3* both increases yield and facilitates recruitment of *sh4* and *qSH3*, which synergistically augment yield, leading to a stepwise model for rice domestication.

The selection of naturally occurring variations in wild plants that provide useful agronomic traits is an essential step in crop domestication. These traits are often related to yield, such as seed number, seed size, and a loss of seed dispersal, or to 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 cultivation. Among several domestication-related traits, the suppression of seed shattering is reported to be the most important genetic change that allowed humans to increase harvestable grain yield differentiating domesticated from wild plants (1, 2). However, the role of visible morphological changes in facilitating domestication is debated.

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 (*Oryza rufipogon* Griff.) was transformed by changes in plant and panicle architecture as well as by acquiring non-seed-shattering behavior. Investigations of 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 seed-shattering loci, *sh4* and *qSH1*, were identified as essential to rice domestication (4, 5). The mutation at *sh4* was largely viewed as the principal genetic change that led to rice domestication. Quantitative trait locus (QTL) analysis identified *sh4* as the locus responsible for the difference in seed-shattering behavior between an accession of wild rice *Oryza nivara* (an annual form of *O. rufipogon*) and *O. sativa* ssp. *indica*. A single-nucleotide polymorphism (SNP) at *sh4* was found to affect the function of the trihelix transcription factor due to an amino acid change in its DNA-binding domain, resulting in suppression of seed shattering (4) (*SI Appendix*, Table S1). *qSH1* was also detected as the QTL largely responsible for the difference in seed-shattering degree between the rice cultivars *O. sativa* ssp. *japonica* and *O. sativa* ssp. *indica*. A SNP of *qSH1* resulted in suppression of seed shattering in *O. sativa* ssp. *japonica* cv. Nipponbare by downregulating

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expression of the downstream gene *OsRIL1* encoding a BEL1-type homeobox family protein (5) (*SI Appendix*, Table S1). Since *sh4* and *qSH1* display large phenotypic variances, and rice cultivars with functional alleles of *sh4* or *qSH1* promote seed shattering, they are recognized as crucial to rice domestication.

To better understand if the domestication process of Asian rice was promoted mainly by a single domestication gene, such as *sh4*, and also to see how *qSH1* and *sh4* are involved in the loss of seed shattering in wild rice, we previously developed introgression lines (ILs) containing small chromosomal segments from *O. sativa* Nipponbare at the *qSH1* and *sh4* loci, referred to as IL(*qSH1*-N) and IL(*sh4*-N), respectively, in the genetic background of wild rice *O. rufipogon* W630 (6). Complete seed shattering was still observed in these lines (6–8), indicating that neither of the single mutations at *qSH1* or *sh4* on its own could account for nonshattering leading to rice domestication. As the *sh4* mutation is commonly observed in cultivated rice, while the *qSH1* mutation is only found in some *japonica* cultivars (5), additional mutation(s) together with *sh4* must have played a role in reducing seed shattering during the early stages of rice domestication. Such quantitatively inherited mutations reducing shattering behavior represent an impediment to selection of shattering loss, which does not occur on the basis of a simple recessive mutation. Thus, it is plausible that the domestication process in rice is more complicated than the previously proposed single domestication allele model.

A change in panicle structure from open to closed may have played a crucial role in mitigating seed-shattering behavior prior to the selection of nonshattering rice (7). *SPR3* is responsible for closed panicle formation and acts as a cis-regulatory element controlling expression of the downstream gene *OsLG1*, which encodes a *SQUAMOSA* promoter-binding protein (7). A closed panicle structure encases the mature seeds, which are retained in the upper part of the panicle due to the long awns present on the lower immature seeds, potentially making these plants more attractive to gatherers. By choosing rice plants with the *SPR3* genetic mutation, gatherers would have increased their collection rate, particularly if the plants with closed panicles also exhibited suppressed seed shattering. In addition, selection of the closed panicle formation also brought about increased self-pollination rates. Therefore, this trait may have potentially contributed to the accumulation and fixation of multiple favorable mutations, leading to the evolution of non-seed-shattering plants thereafter.

In this study, we conducted a genetic analysis of *qSH3*, a 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 also evaluated. We further conducted seed-gathering experiments, which showed how slight genetic changes can significantly impact yields. Finally, we assessed the potential role of closed panicle formation on reducing seed shattering and the relationship between these two distinct traits, seed shattering and closed panicle formation, based on structural mechanical analysis to better understand the selection process toward 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 previously 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 (8) (*SI Appendix*, Fig. S1). IL(*qSH1*-N, *sh4*-N), which contains small chromosomal segments from *O. sativa* Nipponbare at both *qSH1* and *sh4* loci, was crossed with Nipponbare, and the derived F_2 population was subjected to QTL analysis to determine the degree of seed shattering, and the locus *qSH3* was identified (8, 9). High-resolution linkage analysis using a mapping population from a cross between IL(*qSH1*-N, *sh4*-N) and Nipponbare (10) (*SI Appendix*, Fig. S1) identified part of a previously known seed-shattering gene, *OsSh1* (*Os03g0650000*), a homolog of *Sh1* controlling abscission layer formation in sorghum (11) (*SI Appendix*, Figs. S2–S4 and Table S1). 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 (11, 12), but the causal mutation selected during rice domestication was not determined. No significant difference was detected in *qSH3* expression levels in the spikelet base between Nipponbare and W630 (*SI Appendix*, Fig. S4), suggesting that differences in the degree of seed shattering were not caused by changes in *qSH3* expression levels. Among the seven polymorphisms in the region (*SI Appendix*, Fig. S4 and Table S2), only SNP-70, with a SNP of C in W630 and T in Nipponbare, was located in the coding region (exon 1) of *qSH3*. This change caused an amino acid substitution from leucine in W630 to phenylalanine in Nipponbare (8) (*SI Appendix*, Fig. S5 and Table S1).

To test whether SNP-70 is associated with degree of seed shattering, we transformed Nipponbare using two constructs that differed only at the SNP-70 position. In the preliminary experiment using Nipponbare ILs without transformation (Fig. 1*A*), IL(*qSH3*-W), carrying the W630 allele at *qSH3* in the Nipponbare genetic background, showed a non-seed-shattering behavior similar to that of Nipponbare, whereas IL(*qSH1*-W, *qSH3*-W) displayed a significantly higher seed-shattering degree than that of IL(*qSH1*-W) based on the breaking tensile strength (BTS) value (Fig. 1*B* and *SI Appendix*, Fig. S6). These findings indicated that the seed-shattering effect of *qSH3* was considerably enhanced by a functional allele at *qSH1* (*SI Appendix*, Fig. S7). Therefore, we introduced two types of constructs carrying the *qSH3* complementary DNA sequence of W630 ($qSH3^{W630}$) and Nipponbare ($qSH3^{Npb}$) into IL(*qSH1*-W) (Fig. 1*C*). The transgenic plants were expected to express both endogenous *qSH3* of Nipponbare in IL(*qSH1*-W) and $qSH3^{W630}$ or $qSH3^{Npb}$ as the transgene (*SI Appendix*, Fig. S7). Therefore, we screened transformants expressing transgenes using the derived cleaved amplified polymorphic sequences assay targeting a SNP at the 5′ untranslated region, encoded by the W630 promoter region (*SI Appendix*, Figs. S7–S9). As a result, transformants with wild-type $qSH3^{W630}$ showed enhanced seed shattering, compared with the control IL(*qSH1*-W), whereas those with domesticated-type $qSH3^{Npb}$ did not shatter (Fig. 1*D* and *SI Appendix*, Fig. S7), 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 analyzed 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 for nonshattering 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. 2*A*). We further

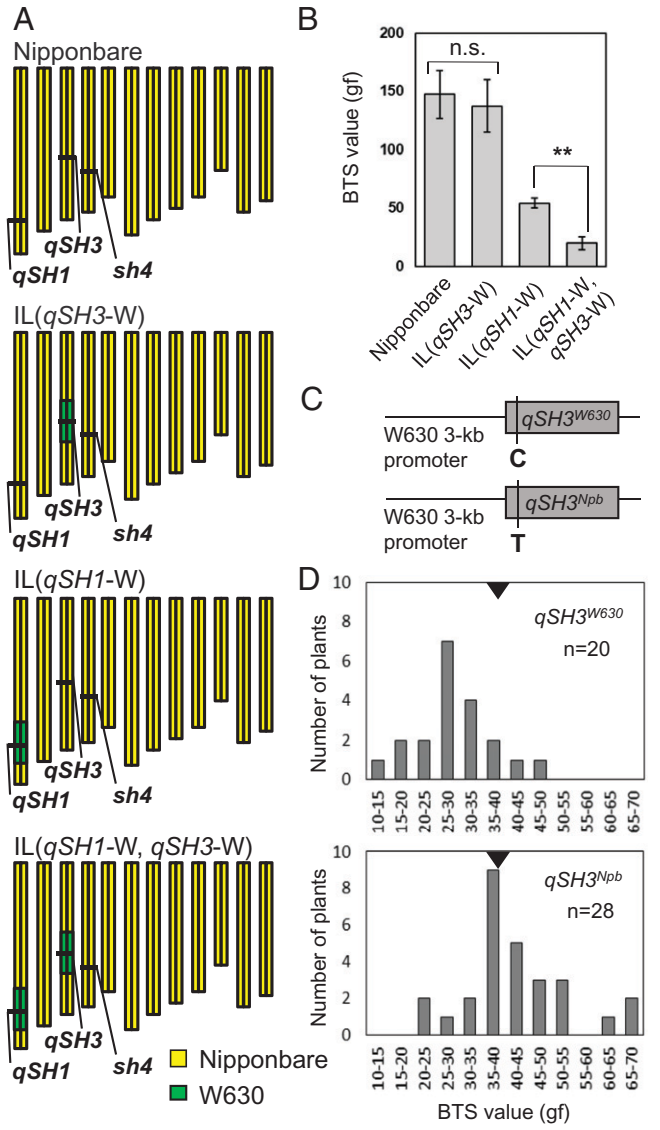

Fig. 1. Identification of a causal SNP of *qSH3* associated with the degree of seed shattering. (*A*) Graphical genotypes of *O. sativa* Nipponbare and ILs for *qSH3* and *qSH1* in the genetic background of cultivated rice *O. sativa* Nipponbare. (*B*) Comparison of seed-shattering degree by BTS values in three ILs, IL(*qSH3*-W), IL(*qSH1*-W), and IL(*qSH1*-W, *qSH3*-W), in the Nipponbare genetic background. Data are mean ± SD of four plants. n.s. and double asterisk (**) indicate not significant and significant at the 1% level based on unpaired Student's *t* test, respectively. (*C*) Two types of constructs carrying *qSH3* complementary DNA 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).

found that the Kasalath haplotype is similar to W630 around the *qSH3* region (*SI Appendix*, Table S2). Using the diverse varieties of the World Rice Core Collection (13), we found that 14 lines, all belonging to *circum*-aus, carried the wild-type functional allele at *qSH3* (*SI Appendix*, Table S3 and Figs. S10 and S11). Furthermore, the Rice 3K genome project data (14) 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. 2*B*). To further understand the footprint of *qSH3* selection, we analyzed nucleotide diversity across the *qSH3* genomic region. Using sequences of the Rice 3K genome collection (14), we detected a selective sweep at *qSH3* in both *indica* and *japonica* but not in the *circum*-aus lineage (Fig. 2*C* and *SI Appendix*, Figs. S12 and S13). These results suggest that *circum*-aus followed a separate trajectory to evolve reduced seed shattering (15). Thus, the reduction in seed shattering is dependent on lineage-specific variations in the subspecies, which is key to understanding the parallel 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 (*SI Appendix*, Table S3), we produced IL(*sh4*-N) and IL(*qSH3*-N) in the genetic background of wild rice *O. rufipogon* W630. Evaluation of the seed-shattering mutations in the wild rice genetic background provides clearer morphological information related to the trait in early rice domestication. Complete formation of the abscission layer similar to that of W630 was observed in both ILs (Fig. 3), confirming that the single mutation at each locus was insufficient for phenotypic change in the abscission layer formation (7, 8). However, a slight inhibition of the abscission layer formation around vascular bundles was observed in IL(*sh4*-N, *qSH3*-N) (Fig. 3). Furthermore, a slight abscission layer inhibition was also observed in several wild rice accessions of *O. rufipogon* carrying mutations at both *sh4* and *qSH3* (*SI Appendix*, Figs. S14 and S15 and Table S4), although they may have gained these

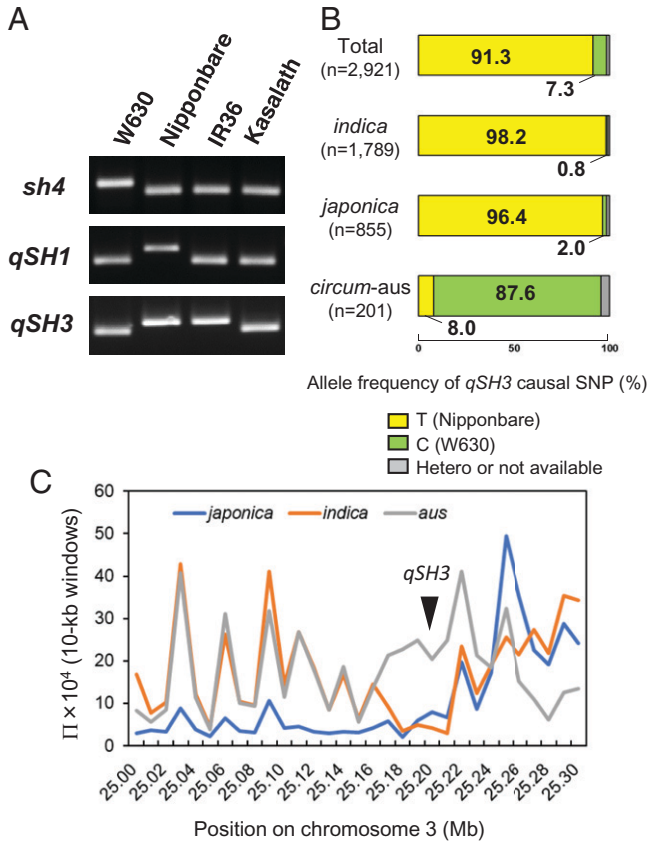

Fig. 2. Lineage-specific selection at the *qSH3* locus in rice. (*A*) Genotyping at *sh4*, *qSH1*, and *qSH3* for *O. rufipogon* W630, *O. sativa japonica* Nipponbare, *indica* IR36, and *circum*-aus Kasalath based on the causal SNPs identified by derived cleaved amplified polymorphic sequence markers. (*B*) Allele frequency of *qSH3* causal SNP (%) in cultivated rice based on the Rice 3K project data. Nipponbare type (T) and W630 type (C) are shown in yellow and green, respectively. (*C*) Nucleotide diversity (π) observed for domesticated rice in the physical position of 25.0 to 25.3 Mb on chromosome 3. In a flanking region of the *qSH3* locus (around 25.2 Mb), π was substantially decreased in *japonica* and *indica* cultivars. π was calculated in 10-kb windows using the SNP data of the Rice 3K project (14).

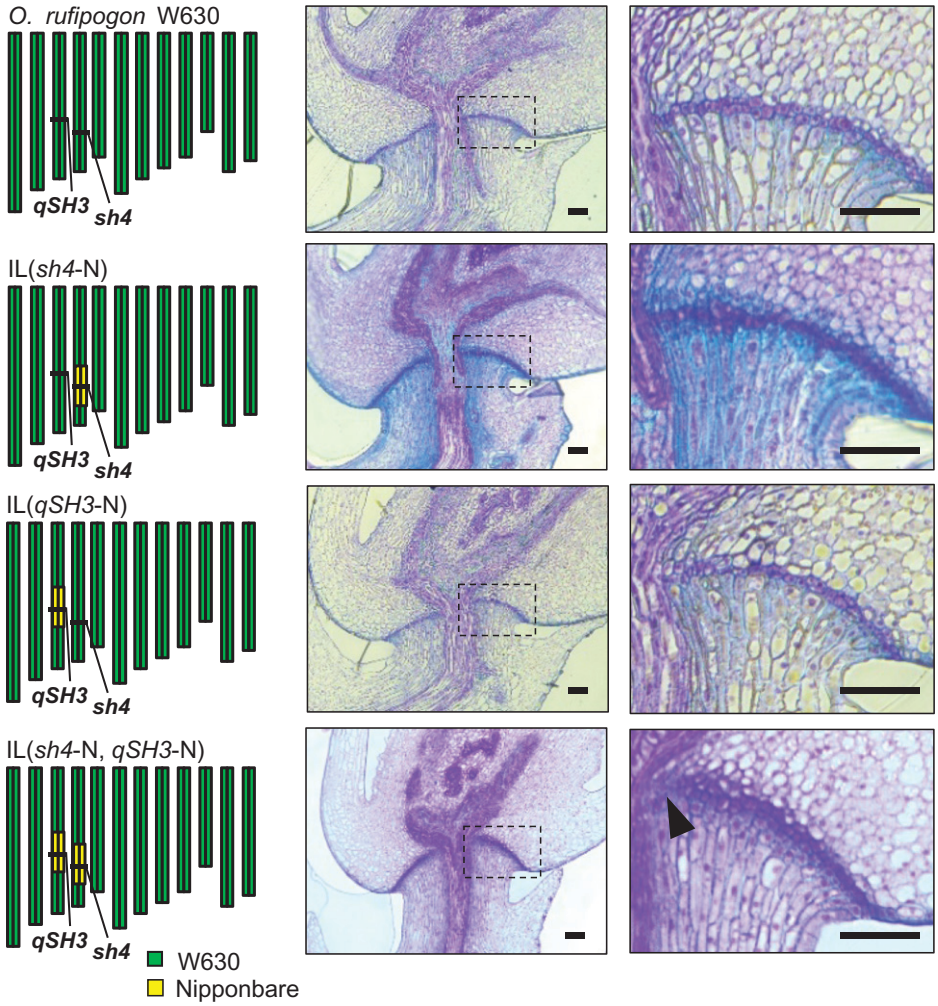

Fig. 3. Abscission layer formation partially inhibited by *sh4* and *qSH3* in wild rice. Graphical genotypes of three ILs in the genetic background of *O. rufipogon* W630 are shown on the left. Abscission layer formations in *O. rufipogon* W630, IL(*sh4*-N), IL(*qSH3*-N), and IL(*sh4*-N, *qSH3*-N) are shown. Each enlarged section indicated by the dotted square is shown in the right-hand panel. A black arrowhead indicates an inhibited area of the abscission layer observed for IL(*sh4*-N, *qSH3*-N). (Scale bars, 100 μm.).

domestic-type alleles through introgressions from cultivated rice (16). Even if the mutations in the two loci displayed a phenotypic difference in the abscission layer formation adequate to reduce shattering under greenhouse conditions without wind, their effect on seed shattering was much less than expected under field conditions.

Seed-Shattering Behavior Associated with a Slight Inhibition of Abscission Layer Formation Was Mitigated by Closed Panicle Formation. Previously, we reported that the closed panicle trait controlled by *OsLG1* had a major effect during rice domestication by facilitating grain harvest (7). In wild rice *O. rufipogon*, as well as other wild rice species, a spreading panicle is characteristic. The primary branch of wild rice starts against the main rachis and gradually bends out before the flowering stage (Fig. 4*A*), reaching nearly a right angle during the seed maturation stage (Fig. 4*B*). The open panicle structure is caused by a bump structure of tissue at the basal parts of primary branches, which is not observed in cultivated rice with closed panicle formation (7). We produced wild rice with the closed panicle trait from cultivated rice and found the trait reduced seed shedding by retaining seeds that get trapped by long awns found on seeds in the lower sections of the panicle. Interestingly, the *SPR3* (a locus regulating *OsLG1*) region is under strong selection in *indica*, *japonica*, and *circum*-aus (*SI Appendix*, Fig. S16), suggesting that the closed panicle trait was selected in the early phase of rice domestication. Thus, closed panicles 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*) (*SI Appendix*, Fig. S17) and compared their morphologies with that of the wild rice *O. rufipogon* W630 (*SI Appendix*, Fig. S18). The heading date of all ILs was similar to that of W630 (*SI Appendix*, Fig. S19), but there was a slight difference in inhibition of abscission layer formation around the vascular bundles depending on the double mutations at *sh4* and *qSH3* (Fig. 3 and *SI Appendix*, Fig. S20) and in open or closed panicle structure depending on *SPR3* mutation (Fig. 4 *A* and *B*). Small BTS values were observed only for IL(*sh4*-N, *qSH3*-N) and IL(*sh4*-N, *qSH3*-N, *SPR3*-N) due to a slight inhibition of abscission layer formation, while the rest were close to zero, as observed in wild rice (Fig. 4*C*). We were interested in assessing the differences in yields brought about by the causal mutations, identifying the combination of alleles that would have provided humans in prehistory with higher yields, and determining 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 (*SI Appendix*, Fig. S21 and Movies S1 and S2). The seed-gathering rates from three ILs with open panicles, namely IL(*sh4*-N),

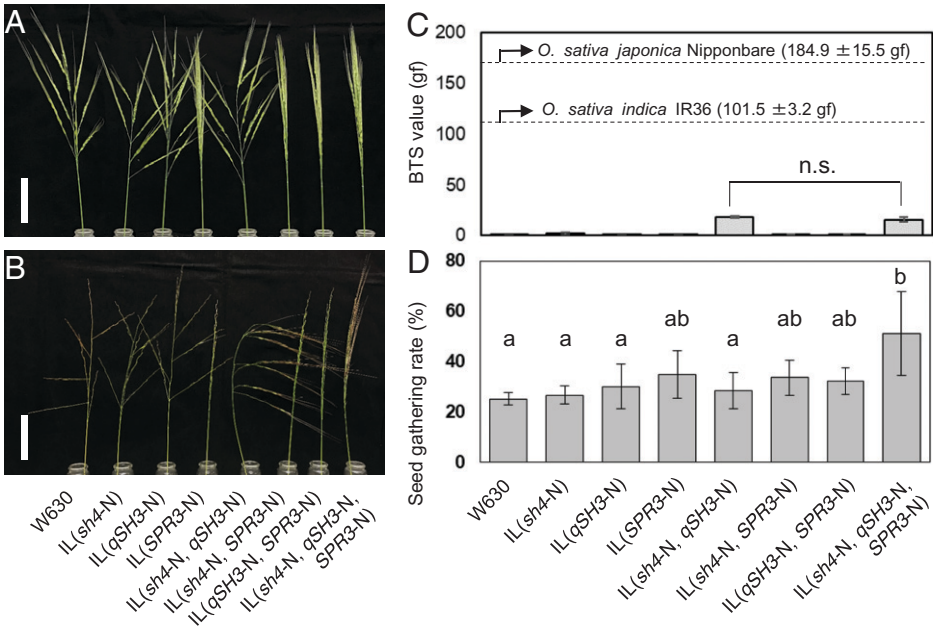

Fig. 4. Role of *sh4*, *qSH3*, and *SPR3* mutations in the initial loss of seed shattering in rice domestication. (*A* and *B*) Panicle shape of the seven ILs at flowering (*A*) and seed-maturation (*B*) stages. (Scale bars, 5 cm.) (*C*) BTS values for the seven ILs. BTS values for *O. sativa japonica* Nipponbare and *indica* IR36 are shown as controls. n.s. indicates not significant by unpaired Student's *t* test. (*D*) Seed-gathering rates (mean ± SD of four plot replicates, with nine plants in each plot) for *O. rufipogon* W630 and the seven ILs. Mean values labeled with different letters are significantly different, whereas those with the same letters are not (Tukey's test with arcsine-transformed values, $P < 0.05$).

IL(*qSH3*-N), and IL(*sh4*-N, *qSH3*-N), were not significantly different from that of W630, regardless of the presence or absence of abscission layer inhibition (Fig. 4*D* and *SI Appendix*, Table S5). However, the three ILs with a closed panicle structure and complete abscission layer formation, namely, IL(*SPR3*-N), IL(*sh4*-N, *SPR3*-N), and IL(*qSH3*-N, *SPR3*-N), presented a slightly increased yield (Fig. 4*D*). In contrast, IL(*sh4*-N, *qSH3*-N, *SPR3*-N), with a combination of a closed panicle structure and slight abscission layer inhibition, showed a significant increase in gathering rate compared with W630 (Fig. 4*D* and *SI Appendix*, Table S5). As closed panicles would be easily visible in the field, humans could have targeted this higher-yielding rice (*SI Appendix*, Fig. S22), but even indiscriminate gathering also would retain larger proportions of grains from closed panicles.

When plants were sown from harvests, closed panicles and reduced seed shattering would increase, owing to the enhanced gathering rate that would occur in the presence of all three domesticated-type alleles. As these alleles increased in frequency and became fixed, yields would have increased, which would have encouraged further investment in rice cultivation (17). The selection for closed panicles instigates self-pollination behavior owing to the long awns, which disturb the free exposure of anthers and stigmas (stigmas and closed panicles) (7). Thus, a closed panicle may also be advantageous in mitigating natural variations in seed-shattering loci by reducing outcrossing. Although awns are undesirable in modern cultivated rice, 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 the Panicle Increasing Yield. To better understand how the closed panicle, caused by *SPR3*, and the inhibition of abscission layer formation, caused by *sh4* and *qSH3*, contributed to the initial loss of seed shattering, we analyzed their roles by performing structural mechanics analysis. The awns of wild rice play a pivotal role in seed dispersal in spreading panicles (Fig. 5*A*). We measured the lengths and weights of awns and grains in wild rice *O. rufipogon* W630 (Fig. 5*B*), and using these values, we calculated the sectional force exerted on the spikelet base depending on panicle angles (*SI Appendix*, Fig. S23). The axial and shear forces were slightly increased in a closed panicle compared to an open panicle. However, the bending moment, which is the predominant factor affecting seed dispersal in an open panicle, was considerably reduced in a closed panicle (*SI Appendix*, Fig. S23). A slight inhibition of the abscission layer formation 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 (Fig. 5 *C* 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 (*SI Appendix*, Fig. S23). The value increased exponentially with increasing length 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. 5*E*), 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 early rice domestication by increasing harvest yields. We identified the causal SNP at *qSH3* involved in reduced seed shattering and demonstrated that the previously proposed *sh4* mutation alone could not trigger nonshattering morphology in rice (6, 7, 16). We also explained how early selection of a closed panicle and the resulting mechanics of spikelet retention would have increased yields and facilitated selection for nonshattering. Our results showed that the initial step in rice

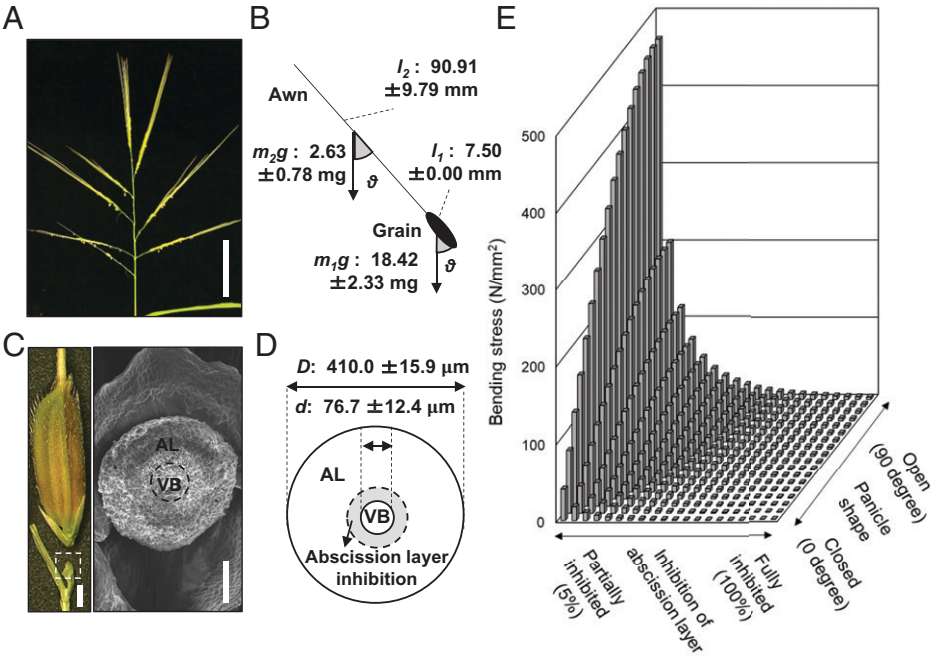

Fig. 5. Structural mechanics analysis of panicle shape and abscission layer inhibition related to the initial loss of seed shattering. (*A*) Panicle shape of *O. rufipogon* W630. (Scale bar, 5 cm.) (*B*) Schematic representation of awn and grain with panicle angle. θ represents the panicle angle. l_1, l_2, m_1g, and m_2g indicate the length of grain, length of awn, weight of grain, and weight of awn, respectively. (*C*) Detachment of grain and pedicel (*Left*), and scanning electron microscopy analysis of the pedicel abscission layer of *O. rufipogon* W630 (*Right*). AL and VB indicate abscission layer and vascular bundle, respectively. (Scale bars, 1 mm [*Left*] and 100 μm [*Right*].) (*D*) Schematic representation of the abscission layer. *D* and *d* indicate the diameters of the abscission layer and vascular bundle, 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. A higher stress was experienced in the open panicle with less inhibited abscission.

domestication was more complex than previously thought. Based on information from our studies, changes are needed from the long-held perspective that the process of rice domestication can be explained by a single domestication allele model to one where synergistic effects of several domestication genes are involved.

The origin and spread of domesticated rice subspecies based on population genetic analysis have been the subject of numerous discussions (15, 18–21). However, most of these studies have been conducted using the genome information of modern cultivars and wild rice, without considering the importance of visible phenotypic changes that could have been targeted by humans. The lineage-specific variations associated with quantitative traits are a key to deepening our understanding of 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. In combination, however, they established an archaic rice that could have been visibly recognized by ancient gatherers as advantageous to increasing yields. The change in harvesting efficiency along with the use of harvesting tools further promoted the selection 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 *SI Appendix*. Primers used for genetic mapping, gene expression analysis, production of transgenic plants, and genotyping are shown in *SI Appendix*, Tables S6–S8.

Data Availability. All study data are included in the article and/or *SI Appendix*.

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