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Deletion in a Quantitative Trait Gene *qPE9-1* Associated With Panicle Erectness Improves Plant Architecture During Rice Domestication

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ABSTRACT

Rice plant architecture is an important agronomic trait and a major determinant in high productivity. Panicle erectness is the preferred plant architecture in *japonica* rice, but the molecular mechanism underlying domestication of the erect panicle remains elusive. Here we report the map-based cloning of a major quantitative trait locus, *qPE9-1*, which plays an integral role in regulation of rice plant architecture including panicle erectness. The R6547 *qPE9-1* gene encodes a 426-amino-acid protein, homologous to the keratin-associated protein 5-4 family. The gene is composed of three Von Willebrand factor type C domains, one transmembrane domain, and one 4-disulfide-core domain. Phenotypic comparisons of a set of near-isogenic lines and transgenic lines reveal that the functional allele (*qPE9-1*) results in drooping panicles, and the loss-of-function mutation (*qpe9-1*) leads to more erect panicles. In addition, the *qPE9-1* locus regulates panicle and grain length, grain weight, and consequently grain yield. We propose that the panicle erectness trait resulted from a natural random loss-of-function mutation for the *qPE9-1* gene and has subsequently been the target of artificial selection during *japonica* rice breeding.

THE worldwide explosion of the human population necessitates an increase in grain yield, which poses a substantial challenge (Rosegrant and Cline 2003). Improvement of plant architecture is considered as a viable approach to increase grain yield, because crop plants with desirable architecture are able to produce much higher yields (Wang and Li 2008). The most striking example arose in the late 1950s, when selection for the semi-dwarf stature in rice and wheat greatly improved plant architecture and yield potential (Peng *et al.* 1999; Monna *et al.* 2002; Sasaki *et al.* 2002; Spielmeyer *et al.* 2002). Tiller, panicle, and leaf morphology also play important roles in shaping high-yield crop architecture. Most plant architecture traits are controlled by quantitative trait loci (QTL) derived from naturally occurring allelic variation. Rice (*Oryza sativa* L.) is the most important food crop in the world (White 1994). It is the staple of diet for heavily populated Asian countries as well as many African countries. Numerous QTL or major genes controlling plant architecture traits have been identified and several have recently been cloned (Li *et al.* 2004; Ashikari *et al.* 2005; Fan *et al.* 2006; Xie *et al.* 2006; Song *et al.* 2007; Yu *et al.* 2007; Jin *et al.* 2008; Shomura *et al.* 2008; Tan *et al.* 2008; Xing *et al.* 2008; Xue *et al.* 2008). Cloning and functional characterization of these genes not only addresses fundamental questions in plant development, but also facilitates bridging the gap between gene identification and breeding application by improving the precision and efficiency of selection.

Rice panicle architecture not only contributes to grain yield, but also to the ecological conditions of cultivated populations and the physicochemical properties of different varieties (Xu *et al.* 1996; Yuan 1997; Chen *et al.* 2001). Presently, most *japonica* rice varieties cultivated in China exhibit the panicle erectness (PE) type of inflorescence (Zhang *et al.* 2002b). PE varieties typically bear short, erect panicles and leaves, which benefit ventilation and light penetration. As a result, populations of PE varieties show higher photosynthetic rates and material production capacity (Liu *et al.* 2001; Zhang *et al.* 2002a; Chen *et al.* 2007). Additionally, PE rice varieties show increased lodging and fertilizer resistance due to decreased plant height (Xu *et al.* 1995). Therefore, PE is the preferred plant architecture for high-yield *japonica* rice.

Since development of the first rice PE variety, Guihuahuang, in the early 1960s, a large number of PE *japonica* varieties have been released in China, including the most well known, Liaojing 5. PE varieties

Sequence data from this article have been deposited with the GenBank Data Libraries: FJ501956, *qPE9-1* allele of R6547 (initially named *PAY1*); FJ554569, *qpe9-1* allele of Wuyunjing 8 (initially named *pay1*).

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have increased yield potential compared to panicle drooping varieties and therefore are the preferred. PE serves as the most suitable morphological index, and has subsequently been brought into super high-yield breeding. The development and cultivation of PE varieties is considered the third landmark trait after dwarf and hybrid rice in the history of Chinese rice breeding (Zhang *et al.* 2002b).

The genetic mechanisms controlling PE have received some attention. Initially, PE was reported to be governed by a recessive gene (Zhu and Gu 1979), while other studies suggested a major gene with dominant or additive effects, and polygenic modifications serving to regulate PE (Xu *et al.* 1995; Wang *et al.* 1997). Chen *et al.* (2006) proposed that panicle angle was controlled by two major genes with additive-dominance-epistatic effects and also polygenes with additive-dominance-epistatic influences, using major gene–polygene mixed inheritance models and a joint analysis method. Pedigree analysis of PE varieties indicated that two-thirds of the varieties possessed genes from the Italian Balilla variety and shared a close relationship with Liaojing 5 (Zhang *et al.* 2002b). The dominant *EP* gene was first reported from chromosome 9, between the two SSR markers RM5833-11 and RM5686-23, at a genetic distance of 1.5 and 0.9 cM, respectively (Kong *et al.* 2007). In a previous study, we identified and characterized *qPE9-1*, a major QTL on chromosome 9 responsible for the erect panicle trait using a double-haploid (DH) population derived from a cross between Wuyunjing 8 and Nongken 57 varieties (Yan *et al.* 2007).

However, despite some progresses of the molecular mechanisms governing rice PE, the complexity of the trait results in substantial gaps in our understanding of its regulation. Here we report on a major QTL, *qPE9-1*, which encodes a keratin-associated protein 5-4, regulates rice PE, and plays pleiotropic roles in an array of plant architecture and yield traits.

MATERIALS AND METHODS

Plant materials: The PE variety Wuyunjing 8 was crossed and backcrossed three times with a panicle drooping *indica* variety R6547 to produce an advanced backcross population. The two parents differ significantly in various agronomic traits, particularly in panicle architecture. R6547 exhibits long, drooping panicles and spindled grains, whereas Wuyunjing 8 bears short, erect panicles and round grains. A pair of near-isogenic lines (NILs) for the qPE9-1 locus, designated R6547 (*qPE9-1*) and R6547 (*qpe9-1*), was selected from the BC_3F_6 generation to analyze genetic effects. The flanking markers c15 and H58 (Yan *et al.* 2007) were used to tag the chromosome segment containing the *qPE9-1* locus in every backcross generation. Applying similar methods, NILs with the *japonica* background were developed from the BC_3F_4 generation for comparative analysis using Wuyujing 3 and Wuyunjing 8 as the recurrent parents and R6547 as the donor. Thirteen *indica* varieties, 27 *japonica* varieties, and seven accessions of wild rice species (supporting information, Table S1) were collected for coding sequence analysis. An additional 50 varieties widely grown in China were used for distribution detection by H90 marker analysis (data not shown). These materials were grown and examined under normal field conditions at the experimental field of Yangzhou University, Yangzhou, China.

Phenotype data collection: All panicle traits were measured during the mature stage. The panicle curvature was presented by the angle included between the lines connecting panicle pedestal with panicle tip and the elongation line of stem. For all above traits, more than 10 representative plants of each line and variety in the middle of each plot were sampled, and the main stem panicle of each plant was chosen for trait measurement. Paddy grains were dried naturally after harvesting and stored at room temperature for at least 1 month before testing. Fully filled grains were used for measuring grain length, width, thickness, and weight. Ten randomly selected grains from each plant were lined up lengthwise along a vernier caliper to measure grain length and then arranged by breadth to measure grain width. Grain thickness was determined for each grain individually using a vernier caliper. All the values were averaged and used as the measurements for each plant. Grain weight was calculated on the basis of 100 grains and converted to 1000-grain weight.

Fine-mapping of *qPE9-1*: The BC_3F_2 segregation population (R6547 background) was used to fine map *qPE9-1*. Several BC_3F_1 plants carrying the heterozygous region flanking the *qPE9-1* locus were selected using MAS, and their self-pollinated progeny were used for fine mapping *qPE9-1*. Four hundred twenty-two plants with extreme drooping panicles from the BC_3F_2 segregating population were chosen to screen recombinants. The candidate genes from Wuyunjing 8 and R6547 were sequenced and analyzed. The newly developed molecular markers covering the *qPE9-1* locus were based on *indica–japonica* differences. Primer sequences are provided in Table S2.

RNA extraction and gene expression analysis: Total RNA was extracted from different tissues during the heading stage. RNA extraction followed the Trizol reagent protocol provided by the manufacturer (Invitrogen) with subsequent *DNase*I (TaKaRa) treatment. Approximately 1 μg of total RNA from each sample was used for first-strand cDNA synthesis. RT–PCR and quantitative real-time PCR was conducted to amplify *qPE9-1* transcript using first-strand cDNA. *OsActin* was also amplified as a control. Quantitative real-time PCR was carried out on an ABI 7500 real-time system (Applied Biosystems) with the SYBR Premix Ex Taq system (TaKaRa). Each set of experiments was repeated three times. The relative amount of the *qPE9-1* transcript is presented as $2^{-\Delta CT}$ according to the ΔC_T method described in the real-time PCR Applications Guide. The ΔC_T value was obtained by subtracting the C_T (threshold cycle) number of the *OsActin* gene from that of the *qPE9-1* gene ($\Delta C_T = C_T$*qPE9-1*$-C_T$*OsActin*). The ΔC_T value was converted to the linear form in terms of $2^{-\Delta CT}$ for statistical analysis. Primer sequences are provided in Table S3.

Transgenic analysis: A 2794-bp DNA fragment containing the *qPE9-1* promoter region (1513 bp before ATG) and the entire coding region (1281 bp) from R6547 was cloned into p*CAMBIA1301* to generate a p-*GPE* construct for complementary tests. A p-*gpe* construct containing the *qpe9-1* promoter region (1513 bp before ATG) and the entire coding region (656 bp) from Wuyunjing 8 was also generated.

To generate the RNAi construct p-*RNAi*, a gene fragment of *qPE9-1* was amplified from R6547 cDNA. A hairpin structure with two inverted repeat fragments was subsequently constructed and transferred into the plant binary vector p*1301UbiNOS*, expressing under the control of the maize ubiquitin promoter (Shi *et al.* 2007).

The full coding region of *qPE9-1* was amplified from R6547 cDNA and was inserted into the p*1301UbiNOS* vector to generate an overexpression construct p-*PEOX*. The coding region of *qpe9-1* was also amplified from Wuyunjing 8 cDNA and inserted into the p*1301UbiNOS* vector to generate p-*peox*.

For mutation sites analysis, a clone from the PAC library of Nipponbare genomic DNA named AP005419 was digested with restriction endonucleases *Eco*RI and *Bam*HI. Then a 14.3-kb genomic DNA fragment containing the entire *qPE9-1* coding region and upstream and downstream sequence was purified and inserted into the plant binary vector p*CAMBIA1301* to generate p-*FL*. Concurrently, a 9.8-kb genomic DNA fragment containing only the *qPE9-1* partial coding region and downstream sequence was digested with *Hin*dIII and inserted into p*CAMBIA1301* to generate p-*CK* for comparison analysis.

The *qPE9-1* 1513-bp promoter region was amplified for *qPE9-1* expression pattern analysis. The amplification product was subcloned into p*CAMBIA1301-GUS* to generate the *qPE9-1* promoter–GUS fusion construct.

All constructs were transformed by *Agrobacterium tumefaciens*-mediated transformation (Hiei *et al.* 1994). All transgenic lines were assayed in the second (T_1) or third (T_2) generations. All primer sequences are provided in Table S3.

Subcellular localization: To determine its exact subcellular location, *qPE9-1* cDNA was fused in-frame with GFP into the p*163-GFP* vector to generate *CaMV35S:qPE9-1::GFP*. *CaMV35S::GFP* was used as a control. The expression constructs were transfected into rice Nipponbare protoplasts. The transformed protoplasts were examined using a confocal microscope (Leica TCS SP5 confocal system). Primer sequences are provided in Table S3.

RESULTS

Development of NILs and phenotypic analysis: An advanced R6547 background population was generated to isolate the *qPE9-1* gene for PE. The phenotypic distribution of panicle architecture in the BC_3F_2 population is shown in Figure S1. Our results demonstrated that panicle curvature, panicle length, grain length, and 1000-grain weight were not independently inherited, *i.e.*, shorter panicles with small grains were always associated with erect panicles. A bimodal distribution of panicle curvature in the BC_3F_2 population suggested this trait is controlled by a semidominant QTL (Figure S1, A), which is consistent with the previous studies (Zhang *et al.* 2002a; Jin *et al.* 2003; Chen *et al.* 2008). Panicle and grain length also showed a semidominant distribution model in the BC_3F_2 population (Figure S1, B andC). Because grain length is always associated with plant yield, we also analyzed the 1000-grain weight distribution in the BC_3F_2 population, which exhibited a similar distribution model (Figure S1, D).

A pair of NILs, R6547 (*qPE9-1*) and R6547 (*qpe9-1*), was developed from a BC_3F_6 generation. The NILs possessed nearly all the genetic background of R6547, with the exception of the introgressed fragment. An array of plant architecture and yield traits was compared between the pair of NILs. During the heading stage, panicle curvature in R6547 (*qpe9-1*) was less than R6547 (*qPE9-1*) (Figure 1, A and F). Panicle curvature differences between NILs were more obvious with grain filling. Finally, panicle curvature in R6547 (*qpe9-1*) was 54.8% of that detected in R6547 (*qPE9-1*) (Figure 1F). We observed a substantial decrease in panicle length (−19.7%) and grain length (−13.2%) in R6547 (*qpe9-1*) compared with R6547 (*qPE9-1*) (Figure 1, A–C, and E). We also observed a significant decrease in grain weight (−13.7%) and grain yield per plant (−17.2%) in R6547 (*qpe9-1*) (Figure 1E). Scanning electron microscopy (SEM) showed that the outer glume epidermal cells of R6547 (*qpe9-1*) were shorter than those of R6547 (*qPE9-1*) (Figure 1D). This result suggested that *qPE9-1* may regulate rice cell size.

The additional agricultural traits measured are listed in Table S4. We observed an obvious shorter leaf, uppermost internode and plant height in R6547 (*qpe9-1*) compared with those in R6547 (*qPE9-1*). However, no obvious differences were detected in grain width, grain thickness, the number of spikelets on the main panicle, primary branch and secondary branch. Two pairs of NILs were developed using MAS and two PE *japonica* varieties (Wuyujing 3 and Wuyunjing 8) as the recurrent parents, and similar results were generated (Figure S2 and Table S5). These results demonstrated that *qPE9-1* regulated panicle curvature and an array of other plant architecture and yield traits, and the effect-increasing allele was derived from R6547.

Map-based cloning of *qPE9-1*: Using a total of 422 individuals with extreme drooping panicles from the 2552 BC_3F_2 plants, we finally delimited *qPE9-1* within a ~32-kb window between the S919 and S927 markers (Figure 2A). The 32-kb DNA fragment located on a single PAC clone (AP005419) containing three genes, Os09g26999, Os09g27010, and Os09g27020, in the Nipponbare genome according to the TIGR Rice Genome Annotation Database (Figure 2A). Os09g27010 encodes a protein kinase APK1B; Os09g27020 an unclassified retrotransposon protein; and Os09g26999 encodes a protein consisting of threeVon Willebrand factor type C [VWFC] domains, one transmembrane domain, and one 4-disulfide-core domain. The VWFC domains are also present in the OVATE protein and GS3 protein, which have been associated with tomato fruit shape and rice grain length regulation, respectively (Liu *et al.* 2002; Fan *et al.* 2006). Os09g26999 demonstrated obvious genetic effects on panicle and grain length, therefore the gene was considered a strong candidate for *qPE9-1*.

To validate Os9g26999 as a candidate gene, we carried out a functional complementary test. The p-*GPE* construct containing the promoter and entire coding region of *qPE9-1* allele from R6547 was transformed into recipient Wuyunjing 8. All plantlets regenerated from the p-*GPE* transformed calli were confirmed positive, and the transgenic lines were further identified. All lines showed a significant increase in panicle curvature compared with the control and exhibited a drooping panicle (Figure 2, A and E). We also observed a significant increase in panicle length and 1000-grain weight

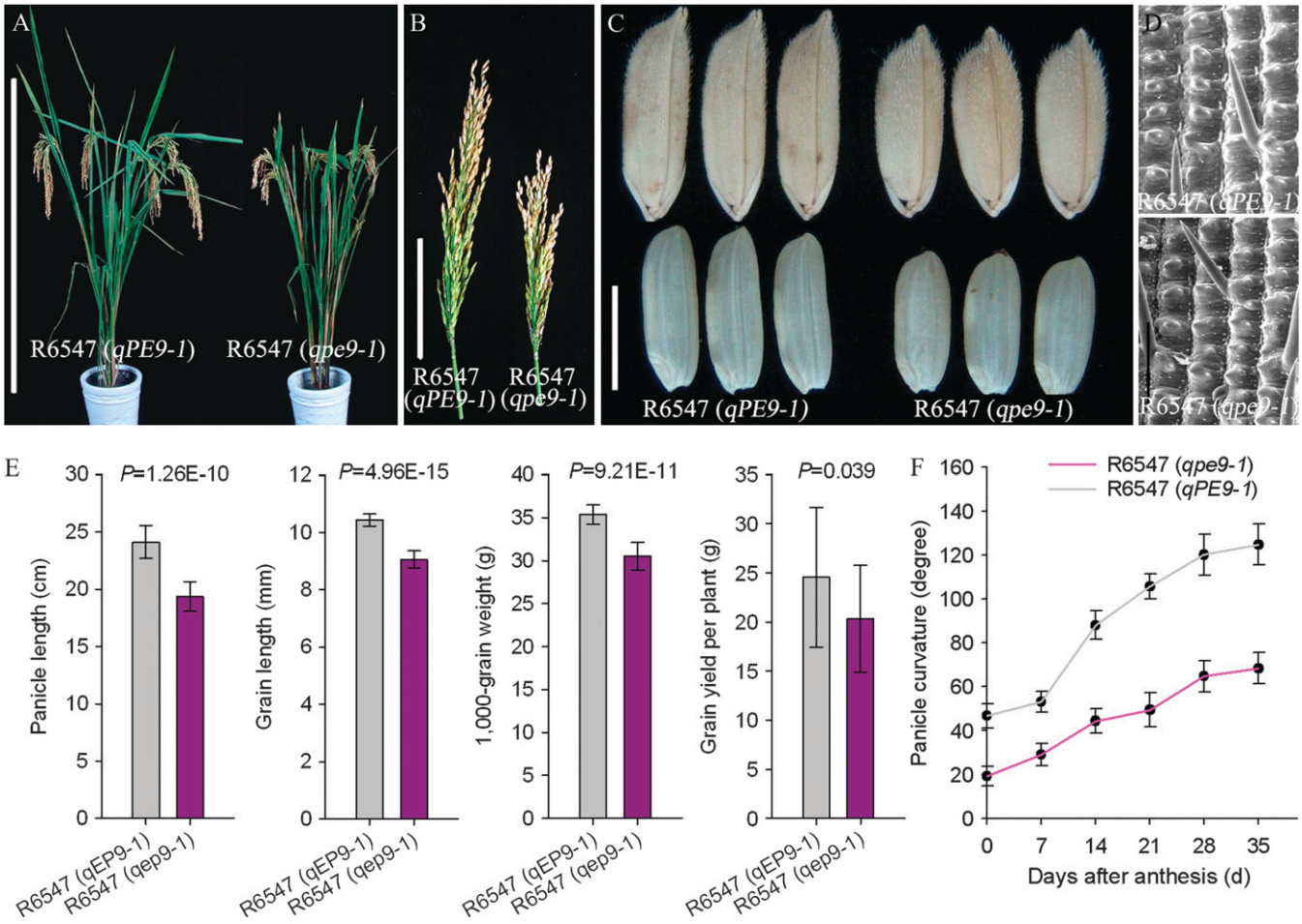

FIGURE 1.—Performance of lines R6547 (*qPE9-1*) and R6547 (*qpe9-1*). (A) Plant phenotype of lines R6547 (*qPE9-1*) and R6547 (*qpe9-1*). Bar, 100 cm. (B) Main panicle of lines R6547 (*qPE9-1*) and R6547 (*qpe9-1*). Bar, 10 cm. (C) Grains and brown rice of lines R6547 (*qPE9-1*) and R6547 (*qpe9-1*). Bar, 5 mm. (D) Scanning electron microscopy (SEM) of rice glume epidermis from lines R6547 (*qPE9-1*) and R6547 (*qpe9-1*). Bar, 200 μm. (E) Comparison of panicle length, grain length, 1000-grain weight, and grain yield per plant between lines R6547 (*qPE9-1*) and R6547 (*qpe9-1*). (F) Dynamic change of panicle curvature after anthesis. Data are means ± SD (n = 10–15). A Student's t-test was applied to generate P-values.

(Figure 2, B–E). Furthermore, compared with the control, grain yield per plant increased in transgenic lines (Figure 2E). The p-*gpe* construct containing the promoter and entire coding region of *qpe9-1* allele from Wuyunjing 8 was simultaneously transformed into Zhonghua 11 (an easy regenerated *japonica* variety with a drooping panicle). But we observed no change in the transgenic lines (data not shown). These results demonstrated that in R6547, Os9g26999 is a key gene (*qPE9-1*) for PE and has pleiotropic effects controlling plant architecture and yield traits and the *qpe9-1* in Wuyunjing 8 is a loss-of-function allele.

Sequence analysis and natural variation of *qPE9-1*: Alignment of *qPE9-1* cDNA with its genomic DNA revealed that *qPE9-1* contained five exons and four introns, encoding a protein of 426 amino acid residues (Figures 2A and 3). FASTA analysis indicated that the *qPE9-1* protein is homologous to the keratin-associated protein (KAP) 5-4 family in human. Results further established that *qPE9-1* contains three VWFC domains (residues 99–153, 276–316, and 339–385), one transmembrane domain (residues 88–106), and one 4-disulfide-core domain (residues 153–166) (http://www.ebi.ac.uk/InterProScan/) (Figure 3). An additional gene controlling grain size (*GS3*) has been identified in rice and also carries VWFC and transmembrane domains (FAN *et al.* 2006), demonstrating the importance of these structures. The fact that the two QTL exhibiting similar protein domains and genetic effects suggested a similar molecular mechanism controls grain size. Thirteen single nucleotide polymorphisms (SNP1–SNP13) and four insertion–deletion polymorphisms (InDel1–InDel4) were detected on the *qPE9-1* locus between R6547 and Wuyunjing 8 (Figure S3). All sequence polymorphisms were delimited in non-coding regions, with the exception of SNP13 and InDel4. SNP13 results in a cystine-to-tyrosine substitution at site 105 (C105Y); and the Wuyunjing 8 allele, due to its InDel4 (637-bp deletion and 12-bp insertion) in exon 5, encodes a presumably truncated protein that lacks 231 C-terminal residues (Figure 3 and Figure S3). The missing

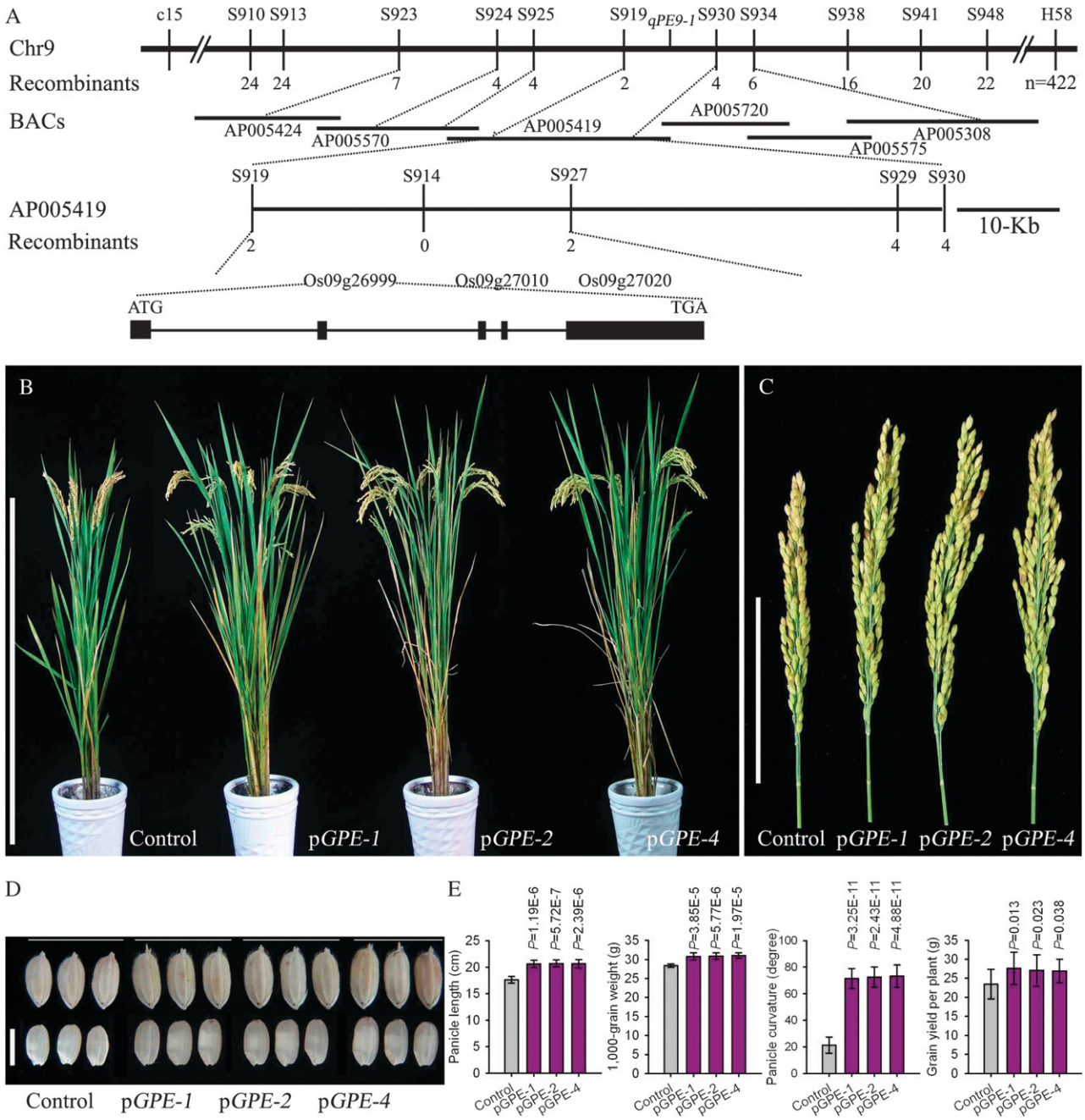

FIGURE 2.—Map-based cloning of *qPE9-1*. (A) The *qPE9-1* gene was finally delimited to a 32-kb genomic DNA region between S919 and S927, and cosegregated with S914. Numbers represent recombination events. Three candidate genes were located within this region in the Nipponbare genome according to the TIGR Rice Genome Annotation Database, one of which was *qPE9-1*. The gene structure of *qPE9-1* is indicated. (B) Phenotypic characters of p*GPE1* lines and control. Bar, 100 cm. (C) Main panicle phenotypes in p*GPE1* lines and control. Bar, 10 cm. (D) Grains and brown rice in p*GPE1* lines and control. Bar, 5 mm. (E) Panicle length, 1000-grain weight, panicle curvature, and grain yield comparisons per plant between p*GPE1* lines and control. Panicle curvature was detected 28 days after anthesis. Data are means ± SD ($n = 10$–20). A Student's *t*-test was applied to generate *P*-values.

C-terminal amino acids cover the two rear VWFC domains (Figure 3), rendering the truncated protein nonfunctional.

Mutation sites analysis: Two mutations are present in rice PE varieties and we were interested which mutation is associated with rice PE. Therefore, we obtained the *qPE9-1* allele DNA sequence from Nipponbare, a famous panicle drooping *japonica* variety. Sequence analysis indicated that Nipponbare and Wuyunjing 8 only differed at InDel4 in the *qPE9-1* coding region (see below). We generated a p-*FL* construct, which covered the entire coding region of the upstream and downstream Nipponbare allele sequence, and transformed it into Wuyunjing 8 (Figure 4A). The p-*CK* construct

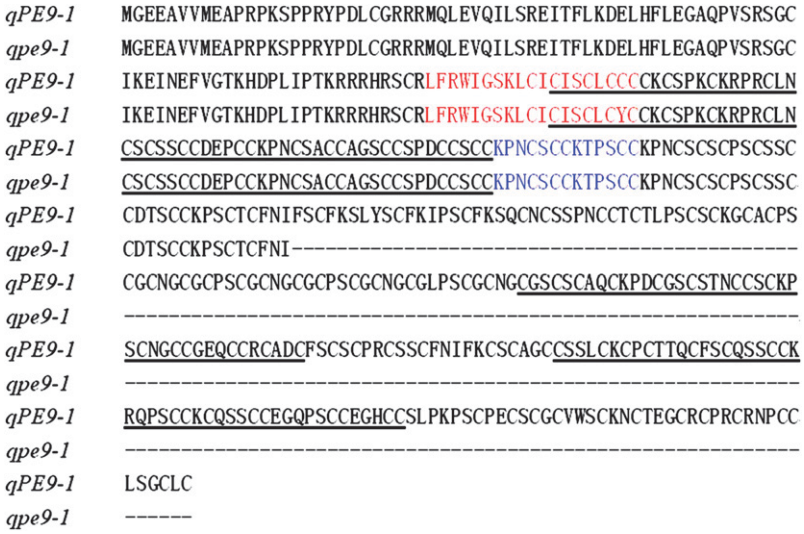

FIGURE 3.—Predicted sequences and structure of the *qPE9-1/qpe9-1* protein in R6547 and Wuyunjing 8. Predicted sequence analysis of the *qPE9-1* protein revealed several known regions and domains (http://www.ebi.ac.uk/InterProScan/). The amino acids marked with black lines indicate the VWFC domains, the amino acids marked blue showed the 4-disulfide-core domain, and the predicted transmembrane domain was marked red.

containing a partial coding region and three-flanking region of the *qPE9-1* gene was used for comparative analysis (Figure 4A). The complementary test showed image results of all 18 p-*FL* independent transgenic lines consistent with that of p*GPE* lines (Figure 4, B–E). In contrast, transgenic plants carrying a p-*CK* construct showed no noticeable change in panicle and plant architecture (Figure 4). On the basis of these results, we concluded that the Nipponbare and R6547 *qPE9-1* alleles are functional and the premature stop codon resulting from InDel4 is responsible for PE.

RNAi and overexpression experiments: Transgenic plants expressing different *qPE9-1* levels were also generated in our study. All 18 transgenic Zhonghua 11 lines carrying p-*RNAi* showed reduced expression levels and exhibited typical erect and short panicles, and a significant decrease in 1000-grain weight (Figure S4 and Figure S5). All 16 transgenic Zhonghua 11 lines carrying p-*PEOX* showed increased expression levels and displayed the opposite phenotype (Figure S4 and Figure S5). In contrast, both transgenic Zhonghua 11 plants expressing p-*peox* and Wuyunjing 8 plants carrying p-*RNAi* showed no obvious change in plant and panicle architecture (data not shown). Taken together, we conclude that *qPE9-1* acts as a functional allele and its loss-of-function mutation leads to PE. These results also implied that PE is a comprehensive trait resulting from short panicles, small grain length, and reduced weight.

Expression pattern and subcellular localization of *qPE9-1*: *qPE9-1* expression pattern was analyzed using transgenic plants expressing the β-*glucuronidase* (GUS) reporter gene under the control of the *qPE9-1* gene promoter region (Figure 5). GUS activity was detected mainly in elongating and dividing tissues, including the shoot apical meristem, and the divisional and elongating zones of stems and knots (Figure 5C). GUS activity was also detected in panicle, sheath, leaf, and root tissues (Figure 5, A, B, D, and E). Quantitative real-time PCR analysis was consistent with GUS staining (Figure 5F). Differences in *qPE9-1* expression levels between R6547 (*qPE9-1*) and R6547 (*qpe9-1*) lines were not observed, suggesting that the genomic sequence changes did not affect expression. Subcellular localization of the *qPE9-1* protein was identified using the chimerical fusion protein *CaMV35S:qPE9-1::GFP*, and *CaMV35S::GFP* alone was used as a control. Confocal microscopy showed that transient expression of the *qPE9-1::GFP* fusion protein in rice protoplast was located in the membrane (Figure 5, J–L). However, expression in the control was distributed throughout the entire cell (Figure 5, G–I). These results suggested that the qPE9-1 protein is a membrane protein.

The deletion associated with the PE trait defines a domestication-related gene: The predicted *qPE9-1* coding region was sequenced for 13 *indica* and 27 *japonica* rice varieties and seven accessions of wild rice (Table S1). Comparison of the predicted coding sequences showed that exclusive of the two mutation sites (SNP13 and InDel4) discussed above, two new mutation sites, SNP14 and SNP15, located in the InDel4 region were detected in the fifth exon. SNP14 (A to T) resulted in an amino acid change (histidine to leucine) and SNP15 (A to T) replaced serine by cystine. Sequence analysis revealed that only PE varieties, including Guihuahuang and its donor parent Balilla, carried the common *qpe9-1* allele of Wuyunjing 8. Most *indica* rice varieties possessed the *qPE9-1* allele in common with R6547, and wild rice accessions shared the other alleles. Four *japonica* varieties with drooping panicles (including Nipponbare and Zhonghua11), shared an allele similar to *qPE9-1* in R6547 (Table S1). Furthermore, a gene-tagged marker H90 anchoring the InDel4 region (YAN *et al.* 2007) was used to examine the distribution of *qPE9-1/qpe9-1* in 18 panicle drooping varieties and 32 PE varieties. All drooping panicle varieties carried *qPE9-1*, while all PE varieties carried *qpe9-1* (data not shown). The single

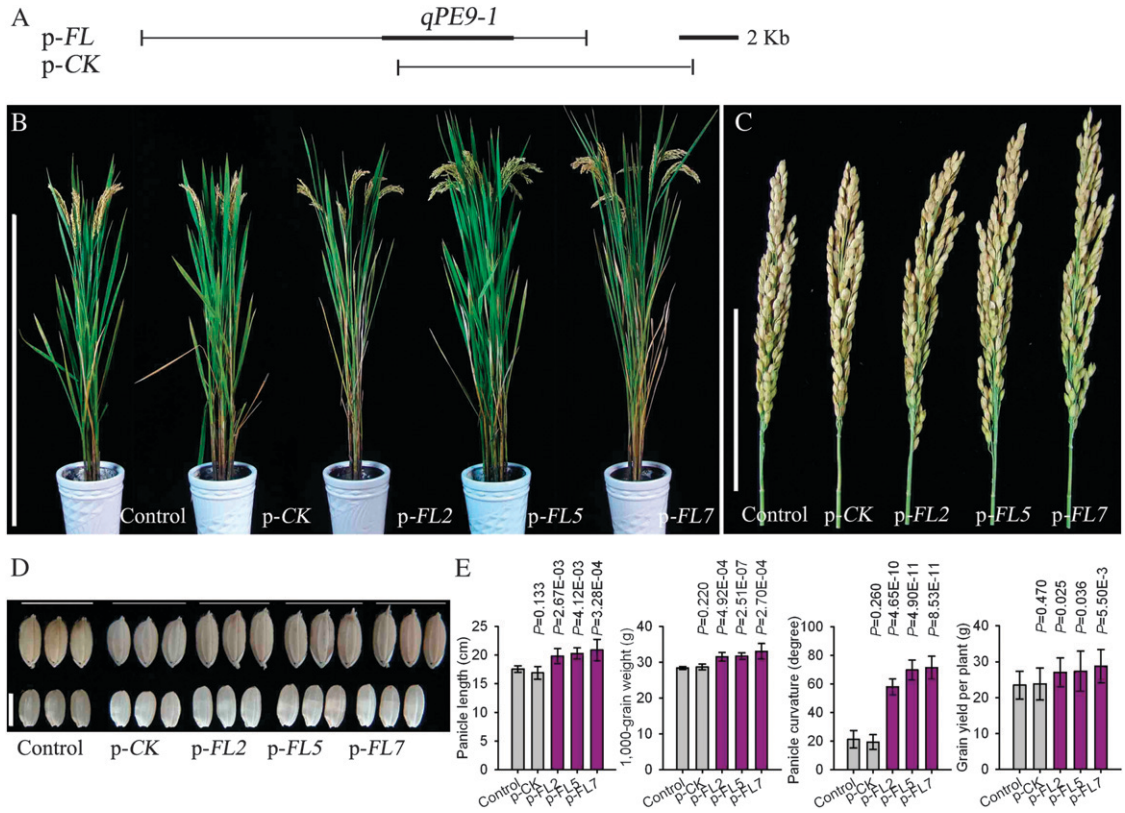

FIGURE 4.—Mutation sites analysis. (A) Genomic fragments containing the entire or partial *qPE9-1* allele from the Nipponbare genomic PAC were cloned into a p*CAMBIA1301* vector to generate p-*FL* and p-*CK* vectors. The p-*CK* was used for comparison and contained only a partial coding and three-flanking region of the *qPE9-1* allele. (B) Phenotypes of transgenic plants and control. Bar, 100 cm. (C) Panicle phenotype of transgenic plants and control. Bar, 10 cm. (D) Grain and brown rice of transgenic plants and control. Bar, 5 mm. (E) Comparison of panicle length, 1000-grain weight, panicle curvature, and grain yield per plant of transgenic plants and control. Panicle curvature was detected 28 days after anthesis. Data are means ± SD ($n = 10$–20). A Student's *t*-test was applied to generate *P*-values.

allele in PE varieties supported strong selection at the *qpe9-1* locus during rice domestication. The *qpe9-1* locus may have arisen from a naturally occurring mutation and was conserved due to its preferred phenotype, similar to *sd1* in *indica* rice.

DISCUSSION

***qpe9-1* is a key gene involved in rice PE formation:** Crop morphological traits are closely associated with yield potential. Idealized plant architecture with a specific combination of morphological traits deemed favorable for photosynthesis, growth, and grain yield was defined by DONALD (1968). In Japan, all cultivated *japonica* varieties bear a drooping panicle, including the most famous variety Nipponbare. However in China, *japonica* PE varieties have become predominant. In cultivated populations of PE varieties, individual competition is reduced to a minimum. PE is considered as high-yielding plant architecture in *japonica* rice due to panicle and plant architecture that significantly optimizes canopy structure (LIU *et al.* 2001; ZHANG *et al.* 2002a; CHEN *et al.* 2007). In the present study, we identified and characterized a major panicle and plant architecture QTL designated *qPE9-1*. Our results provided strong evidence that a deletion in *qPE9-1* leads to PE in rice and has pleiotropic effects on an array of rice traits, including shortened panicle, reduced grain length, and weight. SEM analysis indicated that *qPE9-1* may regulate rice cell size.

Complex traits such as PE are based on naturally occurring variations governed by several genes at quantitative trait loci and their interactions with other genomewide loci. Therefore, for accurate QTL analysis, phenotypic differences in nontarget traits should be minimized in mapping populations. Previous studies identified and characterized the PE trait using various mapping populations and several different genetic modes were proposed. However, the majority of these studies evaluated PE in primary mapping populations, which included $F_{2:3}$, doubled haploid lines (DHs), and BC_1F_1. These populations are not suitable for fine mapping or cloning QTL because of excessive genetic background noise, although they are easy to develop. As a result, a lack of congruence in the results of former studies regarding the PE trait is widespread in the

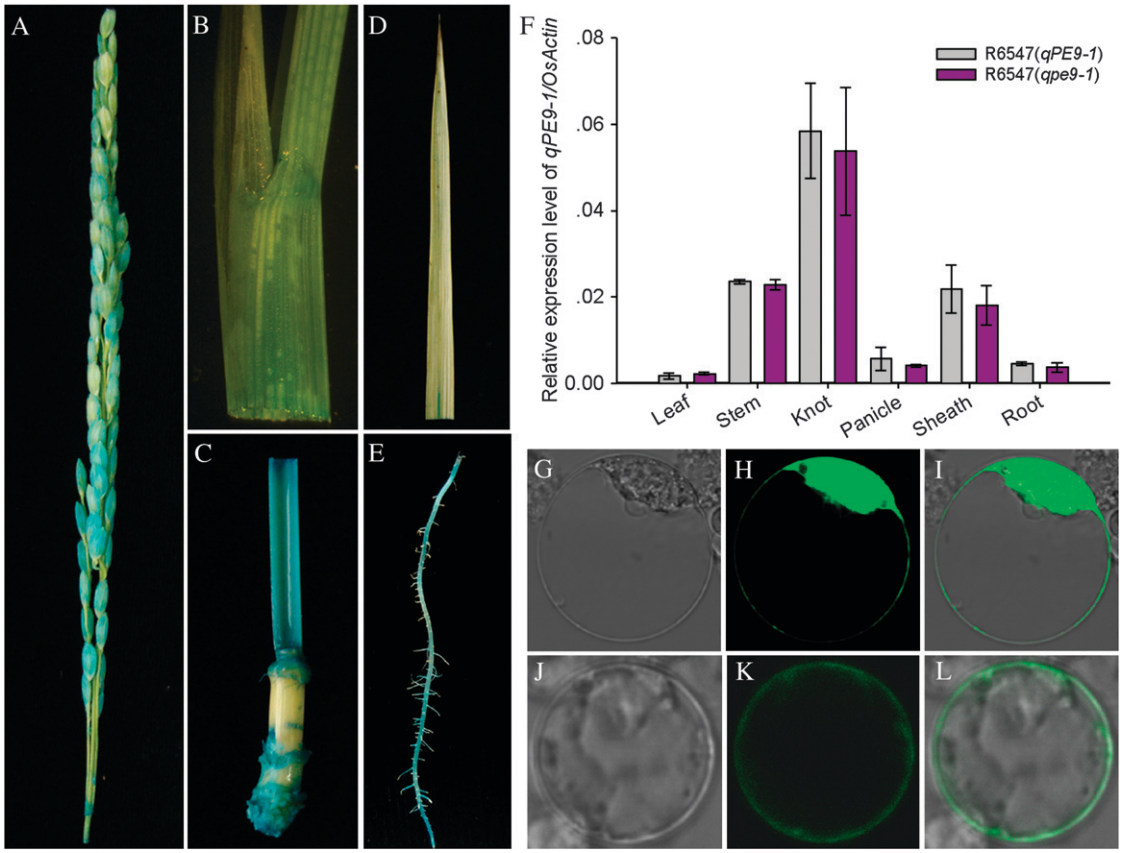

Figure 5.—Expression pattern and subcellular localization of *qPE9-1*. (A) GUS activity in young panicle. (B) GUS activity in sheath. (C) GUS activity in stem and knot. (D) GUS activity in leaf. (E) GUS activity in root. (F) Transcript levels of *qPE9-1* relative to *OsActin* in various tissues detected by quantitative real-time PCR. (G–L) *CaMV35S::GFP* (G–I) and *CaMV35S:qPE9-1::GFP* (J–L) in rice protoplast. The (G and J) photographs were taken in an optic field to examine cell morphology (light), (H and K) were taken in a dark field to localize green fluorescence (GFP), and (I and L) were taken in combination (merge).

literature (Zhu and Gu 1979; Chen *et al.* 2006; Kong *et al.* 2007; Yan *et al.* 2007). To overcome these inconsistencies, we generated three pairs of NILs with varied genetic backgrounds to assure more reliable results. These data together with the transgenic experiments clearly demonstrated that a semidominant gene controls the PE trait and the R6547 and Nipponbare allele is functional.

Gene structure of *qPE9-1*: Our results revealed that the *qPE9-1* encodes a putative homologous gene of keratin-associated protein 5-4 in human. The KAPs form a matrix where intermediate filaments (IFs) are embedded. The complex forms the bulk of keratin fiber, the main structural protein of certain tissues such as hair in humans and wool in animals (Gillespie and Marshall 1980). KAPs fall into three general families; high sulfur proteins, ultrahigh sulfur proteins, and high-glycine-tyrosine proteins in humans. The keratin-associated protein 5-4 is an ultrahigh sulfur protein (Crewther *et al.* 1965; Parry *et al.* 2006). To date, the function of the homologous KAP genes in plants has not been characterized and *qPE9-1* cloning will provide an opportunity to investigate the function of these genes. The *qPE9-1* protein contains three VWFC domains and one transmembrane domain. The VWFC domain has been found in a rice grain size gene *GS3* and the *OVATE* gene in tomato (Liu *et al.* 2001; Fan *et al.* 2006). *GS3* is a negative regulator that prevents increases in grain size and a nonsense mutation in the second exon of the gene results in large grains. *OVATE* determines the conversion of fruit from round to pear shape and is a recessive trait. These results confirmed the wide range of roles for the VWFC domain in fruit/grain shape regulation and also indicated the conserved molecular nature of the domain across species.

The *qpe9-1* was the target of artificial selection during domestication: Balilla, an Italian PE variety, was introduced to China in 1958 and named Beijing 5 (Zhang *et al.* 2002b). Taihu Institute of Agricultural Sciences in Jiangsu Province successfully developed another PE variety, Suzhou 63-2, from the progeny of a natural hybrid of Balilla. Guihuahuang was subsequently developed from Suzhou 63-2 progeny and released into cultivation. In 1974, the first PE variety from Liaoning Province, Qianchonglang, was developed by crossing Balilla with other *indica* and *japonica*

varieties (Yan *et al.* 2007). The famous PE variety Liaojing 5 was developed from Balilla progeny in 1976 and then widely introduced into cultivation. Liaojing 5 displayed high yield potential and was novel for many traits, including panicle and leaf architecture (Zhang *et al.* 2002b). Since then, an increasing number of PE varieties have been developed and released. To date, *japonica* varieties displaying the PE phenotype have been widely cultivated throughout most of the *japonica* growing regions, from Zhejiang to Liaoning Province in China (Yan *et al.* 2007). Zhang *et al.* (2002b) found that two-thirds of the PE varieties in cultivation have genes from Balilla and share a close relationship with Liaojing 5. In this study, we sequenced the *qPE9-1* allele from 13 *indica* and 27 *japonica* varieties, and seven accessions representing different species of wild rice. Our results found that all PE varieties, including Balilla, Guihuahuang, Liaojing 5, and Wuyunjing 8 shared the *qpe9-1* allele, while the panicle drooping varieties possessed the wild-type *qPE9-1* allele. The distribution of the *qPE9-1/qpe9-1* alleles in 18 panicle drooping varieties and 32 PE varieties was examined using a gene-tagged marker, yielding the same results. The single *qpe9-1* allele in all PE varieties confirmed this result and demonstrated that strong selection for the *qpe9-1* locus has occurred during rice domestication.

The breeding value of *qpe9-1*: Our study indicated that all PE varieties analyzed carried the *qpe9-1* allele. The gene confers desirable plant architecture, and was therefore conserved during selection. Furthermore, the gene was found to be a key regulator of plant architecture in the high-yielding *japonica* varieties and serves an important role in PE formation and shaping plant architecture. The *qpe9-1* allele itself is a paradox, conducive to development of the rice architecture, but exhibiting negative effects on individual plant yield. Previous studies also observed that the grain yield per plant of erect panicle type was significantly lower than that of drooping panicle type (Zhou *et al.* 2006; Chen *et al.* 2008). Yield is a complex polygenic trait and difficult to be selected directly, while plant architecture traits are easily observed and readily selected during rice improvement. Although leading to a decrease in grain yield per plant, *qpe9-1* improves plant architecture and population quality in PE *japonica* rice. These qualities provide new insights into the complex relationship between plant architecture and yield. Currently, all *japonica* rice in cultivation is PE varieties. However, PE *indica* varieties are not yet available. Here, we also noted differences between panicle drooping NILs: panicle curvature in the *indica* genetic background NILs was less than that in the *japonica* background NILs. These observations suggested that more than one different gene is responsible for PE in rice subspecies. The *qPE9-1* cloning strategy employed in this study remains a viable approach to isolate other genes determining the PE trait in *indica* rice.

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LITERATURE CITED

Ashikari, M., H. Sakakibara, S. Lin, T. Yamamoto, T. Takashi *et al.*, 2005 Cytokinin oxidase regulates rice grain production. Science **309:** 741–745.

Chen, W. F., Z. J. Xu, L. B. Zhang, W. Z. Zhang and D. R. Ma, 2007 Theories and practices of breeding *japonica* rice for super high yield. Scientia Agricultura Sinica **40:** 869–874.

Chen, W. F., Z. J. Xu, W. Z. Zhang, Z. L. Bu and S. R. Yang, 2001 Creation of new plant type and breeding rice for super high yield. Acta Agronomica Sinica **27:** 665–672.

Chen, X. G., J. B. Liu and D. L. Hong, 2006 Genetic analysis on panicle angle and number of spikelets per panicle by using six generations of three crosses derived from erect×curve panicles in *japonica* rice (*Oryza sativa* L.). Acta Agronomica Sinica **32:** 1143–1150.

Chen, Y. Z., S. L. Wei, C. Liu, W. Y. Zhou, Z. F. Ma *et al.*, 2008 Exploitation of molecular markers for erect panicle trait and study on breeding *Indica* erect panicle varieties in rice. Southwest China J. Agric. Sci. **21:** 6–11.

Crewther, W. G., R. D. B. Fraser, F. G. Lennox and H. Lindley, 1965 The chemistry of keratins. Adv. Protein Chem. **20:** 191–346.

Donald, C. M. 1968 The breeding of crop ideotypes. Euphytica **17:** 385–403.

Fan, C., Y. Xing, H. Mao, T. Lu, B. Han *et al.*, 2006 *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor. Appl. Genet. **112:** 1164–1171.

Gillespie, J. M., and R. C. Marshall, 1980 Variability in the proteins of wool and hair, pp. 67–77 in *Proceedings 6 International Wool Textile Research Conference II*. Pretoria, South Africa.

Hiei, Y., S. Ohta, T. Komari and T. Kumashiro, 1994 Efficient transformation of rice (*Oryza sativa* L.) mediated by Agrobacterium tumeficience and sequence analysis of the boundaries of the T-DNA. Plant J. **6:** 271–282.

Huang, X., Q. Qian, Z. Liu, H. Sun, S. He *et al.*, 2009 Natural variation at the DEP1 locus enhances grain yield in rice. Nat. Genet. **41:** 494–497.

Jin, J., W. Huang, J. P. Gao, J. Yang, M. Shi *et al.*, 2008 Genetic control of rice plant architecture under domestication. Nat. Genet. **40:** 1365–1369.

Jin, X. H., J. Y. Wang, Z. J. Xu, W. Z. Zhang, W. F. Chen *et al.*, 2003 Genetic multiple effects of erect panicle type rice. J. Shenyang Agric. Univ. **34:** 332–335.

Kong, F., J. Y. Wang, J. C. Zou, L. X. Shi, D. M. Jin *et al.*, 2007 Molecular tagging and mapping of the erect panicle gene in rice. Mol. Breed. **19:** 297–304.

Li, J., M. Thomson and S. R. McCouch, 2004 Fine mapping of a grain-weight quantitative trait locus in the pericentromeric region of rice chromosome 3. Genetics **168:** 2187–2195.

Liu, J., J. Van Eck, B. Cong and S. D. Tanksley, 2002 A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc. Natl. Acad. Sci. USA **99:** 13302–13306.

Liu, W., Z. J. Xu, W. F. Chen, J. Q. Li, L. X. Li *et al.*, 2001 Effect of different N-level on canopy photosynthetic characteristics of rice varieties with erect panicle type. J. Shenyang Agric. Univ. **32:** 8–12.

Monna, L., N. Kitazawa, R. Yoshino, J. Suzuki, H. Masuda *et al.*, 2002 Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. DNA Res. **9:** 11–17.

Parry, D. A., T. A. Smith, M. A. Rogers and J. Schweizer, 2006 Human hair keratin-associated proteins: sequence regularities and structural implications. J. Struct. Biol. **155:** 361–369.

Peng, J., D. E. Richards, N. M. Hartley, G. P. Murphy, K. M. Devos *et al.*, 1999 'Green revolution' genes encode mutant gibberellin response modulators. Nature **400:** 256–261.

Rosegrant, M. W., and S. A. Cline, 2003 Global food security: challenges and policies. Science **302:** 1917–1919.

Sasaki, A., M. Ashikari, M. Ueguchi-Tanaka, H. Itoh, A. Nishimura *et al.*, 2002 Green revolution: a mutant gibberellin-synthesis gene in rice. Nature **416:** 701–702.

Shi, Z., J. Wang, X. Wan, G. Shen, X. Wang *et al.*, 2007 Overexpression of rice *OsAGO7* gene induces upward curling of the leaf blade that enhanced erect-leaf habit. Planta **226:** 99–108.

Shomura, A., T. Izawa, K. Ebana, T. Ebitani, H. Kanegae *et al.*, 2008 Deletion in a gene associated with grain size increased yields during rice domestication. Nat. Genet. **40:** 1023–1028.

Song, X. J., W. Huang, M. Shi, M. Z. Zhu and H. X. Lin, 2007 A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat. Genet. **39:** 623–630.

Spielmeyer, W., M. H. Ellis and P. M. Chandler, 2002 Semidwarf (sd-1), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc. Natl. Acad. Sci. USA **99:** 9043–9048.

Tan, L., X. Li, F. Liu, X. Sun, C. Li *et al.*, 2008 Control of a key transition from prostrate to erect growth in rice domestication. Nat. Genet. **40:** 1360–1364.

Wang, B. L., Y. H. Dong and S. Wang, 1997 Studies on genetic activities of semidwar sm and erect-panicle in rice. J. Shenyang Agric. Univ. **28:** 83–87.

Wang, Y., and J. Li, 2008 Molecular basis of plant architecture. Annu. Rev. Plant Biol. **59:** 253–279.

White, P. T., 1994 Rice: the essential harvest. Natl. Geogr. **185:** 48–79.

Xie, X., M. H. Song, F. Jin, S. N. Ahn, J. P. Suh *et al.*, 2006 Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines derived from a cross between *Oryza sativa* and *Oryza rufipogon*. Theor. Appl. Genet. **113:** 885–894.

Xing, Y. Z., W. J. Tang, W. Y. Xue, C. G. Xu and Q. Zhang, 2008 Fine mapping of a major quantitative trait loci, *qSSP7*, controlling the number of spikelets per panicle as a single Mendelian factor in rice. Theor. Appl. Genet. **116:** 789–796.

Xu, Z. J., W. F. Chen, L. B. Zhang and S. R. Yang, 1995 Advance in estimation and utilization of rice erect panicle. J. Shenyang Agric. Univ. **26:** 335–341.

Xu, Z. J., W. F. Chen, H. F. Zhou, B. L. Zhang and S. R. Yang, 1996 The physiological and ecological characters and application prospects of erect panicle rice population. Chinese Sci. Bull. **41:** 1122–1126.

Xue, W., Y. Xing, X. Weng, Y. Zhao, W. Tang *et al.*, 2008 Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. Nat. Genet. **40:** 761–767.

Yan, C. J., J. H. Zhou, S. Yan, F. Chen, M. Yeboah *et al.*, 2007 Identification and characterization of a major QTL responsible for erect panicle trait in *japonica* rice (*Oryza sativa* L.). Theor. Appl. Genet. **115:** 1093–1100.

Yu, B., Z. Lin, H. Li, X. Li, J. Li *et al.*, 2007 *TAC1*, a major quantitative trait locus controlling tiller angle in rice. Plant J. **52:** 891–898.

Yuan, L. P., 1997 Hybrid rice breeding for super high yield. Hybrid Rice **12:** 1–6.

Zhang, W. Z., Z. J. Xu, W. F. Chen, L. B. Zhang, X. H. Jin *et al.*, 2002a The research progress on erect panicle type of rice. J. Shenyang Agric. Univ. **33:** 471–475.

Zhang, W. Z., Z. J. Xu, L. B. Zhang, W. F. Chen, F. L. Qiu *et al.*, 2002b Analysis on evolution for the erect panicle type varieties of rice. J. Shenyang Agric. Univ. **33:** 161–166.

Zhou, W. Y., D. L. Bai, X. Q. Yang, X. Y. Li, Y. F. Liu *et al.*, 2006 Inheritance of erect panicle type and its effect on the agronomic characteristics of *indica* rice. Guangxi Agric. Sci. **37:** 490–493.

Zhu, L. H., and M. H. Gu, 1979 The inheritance of rice grain shattering. Hereditas **1:** 17–19.

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GENETICS

Supporting Information

http://www.genetics.org/cgi/content/full/genetics.109.102681/DC1

Deletion in a Quantitative Trait Gene *qPE9-1* Associated With Panicle Erectness Improves Plant Architecture During Rice Domestication

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DOI: 10.1534/genetics.109.102681

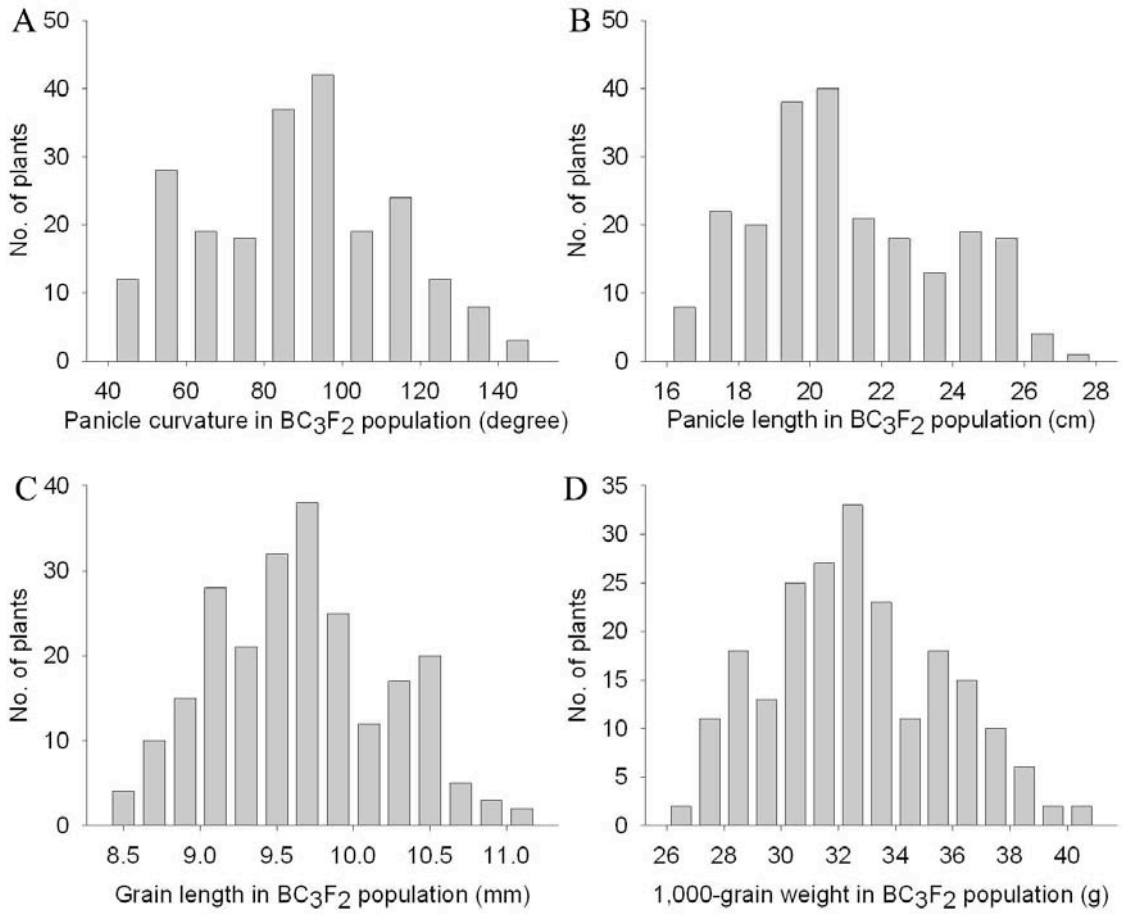


FIGURE S1.—Phenotypic distributions of panicle curvature (A), panicle length (B), grain length (C) and 1,000-grain weight (D) in BC_3F_2 population. Panicle curvature was detected 28 days after anthesis.

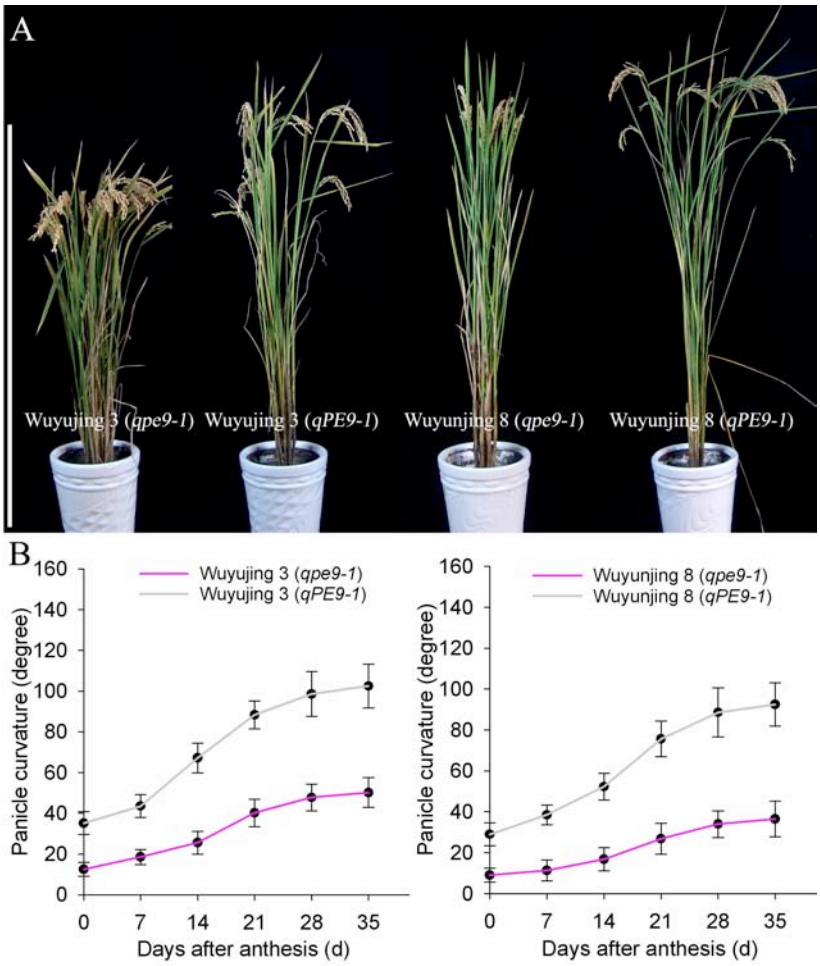
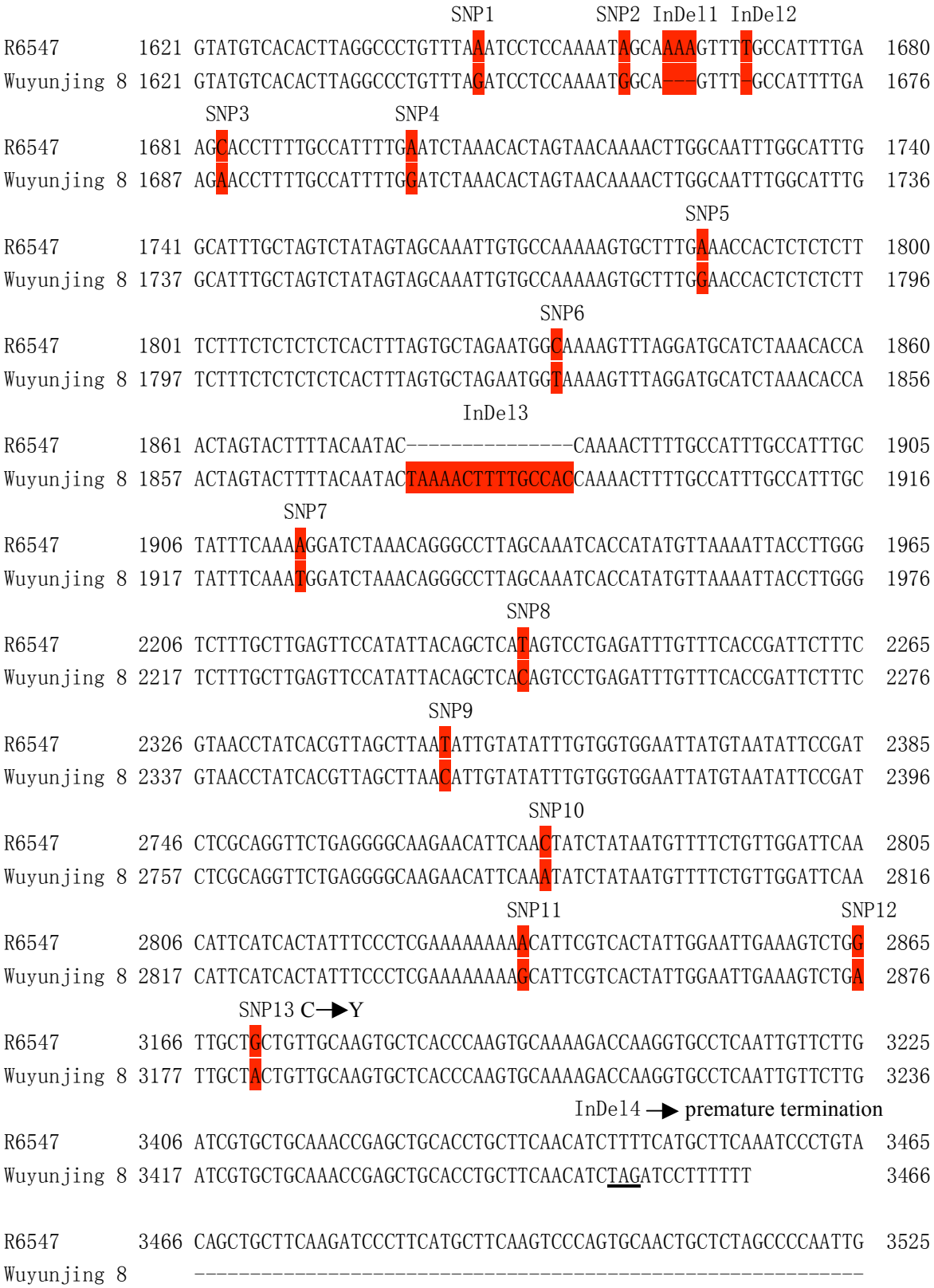


FIGURE S2.—Performance of NILs with Wuyujing 3 and Wuyunjing 8 background. (A) Phenotype of NILs with Wuyujing 3 and Wuyunjing 8 background. Scale bar, 100 cm. (B) Dynamic change in panicle curvature of the two pairs of NILs after anthesis. Data are mean±s.d. (n=10-15).



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R6547        3526 CTGCACTTGCACCCATCCAAGCTGTAGCTGCAAGGGCTGTGCCTGTCCAAGCTGTGGATG  3585
Wuyunjing 8       ------------------------------------------------------------

R6547        3586 CAACGGCTGTGGCTGTCCAAGCTGCGGATGCAACGGTTGTGGCTGTCCAAGCTGCGGTTG  3645
Wuyunjing 8       ------------------------------------------------------------

R6547        3646 CAACGGCTGTGGCCTTCCAAGCTGCGGTTGCAACGGCTGCGGCTCGTGCTCTTGCGCCCA  3705
Wuyunjing 8       ------------------------------------------------------------

R6547        3706 ATGCAAACCCGATTGTGGCTCGTGCTCTACCAATTGCTGTAGCTGCAAGCCAAGCTGCAA  3765
Wuyunjing 8       ------------------------------------------------------------

R6547        3766 CGGCTGCTGCGGCGAGCAGTGCTGCCGCTGCGCGGACTGCTTCTCCTGCTCGTGCCCTCG  3825
Wuyunjing 8       ------------------------------------------------------------

R6547        3826 TAGCTCCAGCTGCTTCAACATCTTCAAATGCTCCTGCGCTGGCTGCTGCTCGAGCCTGTG  3885
Wuyunjing 8       ------------------------------------------------------------

R6547        3886 CAAGTGCCCCTGCACGACGCAGTGCTTCAGCTGCCAGTCGTCATGCTGCAAGCGGCAGCC  3945
Wuyunjing 8       ------------------------------------------------------------

R6547        3946 TTCGTGCTGCAAGTGCCAGTCGTCTTGCTGCGAGGGGCAGCCTTCCTGCTGCGAGGGACA  4005
Wuyunjing 8       ------------------------------------------------------------

R6547        4006 CTGCTGCAGCCTCCCGAAACCGTCGTGCCCTGAATGTTCCTGTGGGTGTGTCTGGTCTTG  4065
Wuyunjing 8       ------------------------------------------------------------

R6547        4066 CAAGAATTGTACAGAGGGTTGTCGATGCCCACGGTGTCGTAACCCATGCTGTCTCAGTGG  4125
Wuyunjing 8  3467 ----------------GGGTTGTCGATGCCCACGGTGTCGTAACCCATGCTGTCTCAGTGG  3511

R6547        4126 TTGCTTATGTTGA                                                 4138
Wuyunjing 8  3512 TTGCTTATGTTGA                                                 3524
```

FIGURE S3.—Sequence comparison between R6547 and Wuyunjing 8 on the *qPE9-1* locus. SNP1-SNP12 and InDel1-InDel3 were detected in a non-coding region; SNP13 and InDel4 were detected in a coding region, resulting in amino acid changes.

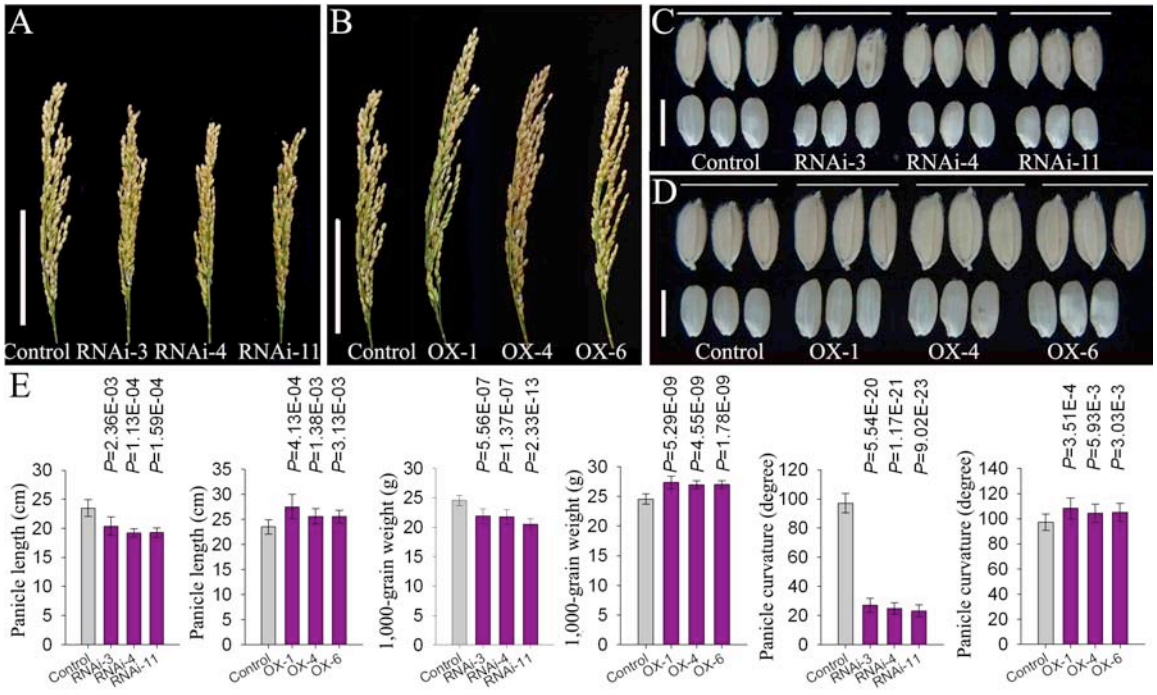

FIGURE S4.—RNAi and over-expression experiments. (A) Main panicles in RNAi lines and control. Scale bar, 10 cm. (B) Main panicles in over-expression lines and control. Scale bar, 10 cm. (C) Grains and brown rice in RNAi lines and control. Scale bar, 5 mm. (D) Grains and brown rice in over-expression lines and control. Scale bar, 5 mm. (E) Comparison of panicle length, 1,000-grain weight and panicle curvature between control and transgenic lines. Panicle curvature was detected 28 days after anthesis. Data are mean±s.d. (n=10-20). A student' *t*-test was used to generate the *P*-values.

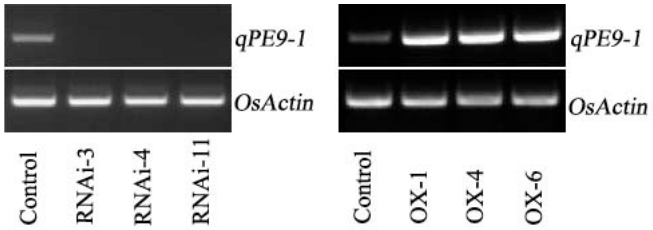

FIGURE S5.—*qPE9-1* expression level in panicles in RNAi (left) and over-expression lines (right) during heading stage detected by RT-PCR. *OsActin* was amplified as the control.

TABLE S1

Plant materials used for sequence analysis

Name	Species	Origin	SNP13	InDel4	SNP14	SNP15	Genotype	Phenotype
W0107	*O. rufipogen*	India	G	Normal	A	A	*qPE9-1*	Drooping
Acc.103827	*O. rufipogen*	Unknown	G	Normal	T	A	Similar to *qPE9-1*	Drooping
Acc.104404	*O. rufipogen*	Unknown	G	Normal	T	T	Similar to *qPE9-1*	Drooping
Acc.104640	*O. rufipogen*	Vietnam	G	Normal	T	T	Similar to *qPE9-1*	Drooping
Dongxiang	*O. rufipogen*	China	G	Normal	T	T	Similar to *qPE9-1*	Drooping
Acc.103849	*O. perennis*	India	G	Normal	T	T	Similar to *qPE9-1*	Drooping
Acc.101937	*O. barthii*	Senegal	G	Normal	T	T	Similar to *qPE9-1*	Drooping
R6547	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
8006	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
9311	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Minghui 72	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Longtefu	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Dular	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Aijiaonante	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Aizaizhan	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Guangchangai	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Guichao 2	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Taizhongzailai 1	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
Minghui 63	*O. indica*	China	G	Normal	A	A	*qPE9-1*	Drooping
IR 24	*O. indica*	Phillipines	G	Normal	A	A	*qPE9-1*	Drooping
Nipponbare	*O. japonica*	Japan	A	Normal	T	T	Similar to *qPE9-1*	Drooping
Zhonghua 11	*O. japonica*	China	A	Normal	T	T	Similar to *qPE9-1*	Drooping
Kuifeng	*O. japonica*	Japan	A	Normal	T	T	Similar to *qPE9-1*	Drooping
Nongken 58	*O. japonica*	Japan	A	Normal	T	T	Similar to *qPE9-1*	Drooping
Balilla	*O. japonica*	Italy	A	InDel	-	-	*qpe9-1*	Erect
Guihuahuang	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
3017	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
3015	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Wuyunjing 7	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Wuyunjing 8	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Wuyujing 3	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Wuxiangjing 9	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Xudao 3	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Xudao 4	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Ruanyu	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Liaojing 5	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Shengnong 265	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Fuhe	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Jingpaifuhe	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect

Zaofeng 9	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Zhengdao 88	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Zhengdao 99	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Ningjing 1	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Xiangjing 111	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Xiangjing 49	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Yujing 6	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect
Huai 68	*O. japonica*	China	A	InDel	-	-	*qpe9-1*	Erect

TABLE S2

Molecular markers newly developed for map-based cloning

Markers	Forward primers (5'-3')	Reverse primers
S910	AGAGGGAATGGACAGATGG	TTTTGGTTTCACTTAGGCTTT
S913	AGCCTATTGTATGACCTCTGC	ATCGTTGCTTTCACCTTCC
S914	CTAAATGAGCGGTAACCTTG	CTTAGTCCACCAAATACCTGA
S919	TTATTGATGAGAACCAAGAAAC	CATTTACTCAGGTTAGCGAC
S923	CCAATCCCAATCAAAGCAG	TACAAAATGTCCCACCCTC
S924	TGACAGCAGGAAAAGAT	ATTGTTGTTCACCAGGC
S925	GCTGAAGGTAGAGGCGTAGG	TCGGTTGAGCAGGGATTG
S927	ACTGGTGGGTCACTCTTAC	ATTCACTCCTGCACTTCTA
S929	CACCAACCTATCCACCTAC	TCTGGGCTTTGCTGAATC
S930	TTCTGACCGAGCAACCG	GTCTACAAGGAGTGGGCA
S934	ACGGAGACACGAGTTATTC	TACTTGGGTGCCCTATTC
S938	ATTAGCACATTTCCCTGG	TTATTTCGCCTCTCACTG
S941	GTCAACCACAACCACCAT	TAAGCGGATTATTAGGCG
S948	TACCGATAACCTCGTCCC	TCTCAGAGCCCACAACAC

TABLE S3

Primers used for functional analysis. Restriction recognition site underlined, and protection bases in italics

Primer name	Primer sequence (5'-3')	Purpose
GPE -1F	*AAAA*GAATTCCATACTACCCGGGGTAGCAGCG	Complementary test
GPE -1R	*AAAA*GGATCCCTCCACACGCAGCACGCCAACG	Complementary test
GPE -2F	*AAAA*GGATCCATGCCCATGAGTGAAGGCGG	Complementary test
GPE -2R	*AAAA*GTCGACTCAACATAAGCAACCACTGAG	Complementary test
gpe -1F	*AAAA*GAATTCCATACTACCCGGGGTAGCAGCG	Complementary test
gpe -1R	*AAAA*GGATCCCTCCACACGCAGCACGCCAACG	Complementary test
gpe -2F	*AAAA*GGATCCATGCCCATGAGTGAAGGCGG	Complementary test
gpe -2R	*AAAA*GTCGACTCAACATAAGCAACCACTGAG	Complementary test
qPE9-1-RNAi-F	*CGC*ACTAGTAGACCAAGGTGCCTCAATT	RNAi
qPE9-1-RNAi-R	*CGC*GGATCCGCATCGACAACCCTCTGT	RNAi
qPE9-1-OX-F	*AAAA*GGATCCGGGGTGGTTCTGAGTTGG	Over-expression
qPE9-1-OX-R	*A AAA*ACTAGTCGGTTCAACCTCGTCTCATA	Over-expression
qpe9-1-OX-F	*AAAA*GGATCCGGGGTGGTTCTGAGTTGG	Over-expression
qpe9-1-OX-R	*A AAA*ACTAGTCGGTTCAACCTCGTCTCATA	Over-expression
qPE9-1-GUS-F	*CGC*GGATCCCATACTACCCGGGGTAGCAGCG	Expression analysis
qPE9-1-GUS-R	*CGC*AAGCTTCTCCACACGCAGCACGCCAACG	Expression analysis
qPE9-1-GFP-F	*AAAA*GTCGACATGCCCATGAGTGAAGGCGG	GFP analysis
qPE9-1-GFP-R	*AAAA*CCATGGACATAAGCAACCACTGAGAC	GFP analysis
RT-*qPE9-1*-F	GGAGGAGGCGGTGGTGAT	RT and Real-time PCR
RT-*qPE9-1*-R	CACCGAAAAAGACGGCAAG	RT and Real-time PCR
OsActin-F	GATGACCCAGATCATGTTTG	RT and Real-time PCR
OsActin-R	GGGCGATGTAGGAAAGC	RT and Real-time PCR
Gene1-F	GCGGCGATTTATACCCAC	Sequencing
Gene1-R	ACGAGGAGCCCAACCAA	Sequencing
Gene2-F	AGCAGGAATCTTTATGGG	Sequencing
Gene2-R	CTAAACAGGGCCTAAGTG	Sequencing
Gene3-F	ATTTGTTTCACCGATTCTTTCC	Sequencing
Gene3-R	ATTGAGGCACCTTGGTCTTT	Sequencing
Gene4-F	TTTCGGTGGATCGGGTAT	Sequencing
Gene4-R	CATTGGGCGCAAGAGC	Sequencing
Gene5-F	AAAGACCAAGGTGCCTCA	Sequencing
Gene5-R	TGGTTCAACCTCGTCTCATA	Sequencing

TABLE S4

Comparison of other agricultural characters between R6547 (*qPE9-1*) and R6547 (*qpe9-1*). Data are means±s.d. (n=10-15)

Traits	R6547 (*qPE9-1*)	R6547 (*qpe9-1*)
Grain width (mm)	3.0±0.1	3.1±0.1
Grain thickness (mm)	2.2±0.1	2.2±0.1
Plant height (cm)	101.8±3.7	87.5±3.8
Internode length (cm)		
-1	33.6±1.9	25.6±2.0
-2	14.8±0.8	14.6±1.0
-3	10.5±5.5	10.4±1.3
Leaf length (cm)		
-1	43.0±5.6	27.5±4.6
-2	53.6±5.6	33.7±3.5
-3	52.9±7.5	38.4±2.4
Leaf width (cm)		
-1	2.9±0.1	3.0±0.2
-2	2.3±0.2	2.5±0.2
-3	2.1±0.1	2.3±0.2
No. Spikelets on the main panicle	303.0±40.9	288.1±44.6
No. primary branch	16.8±2.2	16.7±1.4
No. secondary branch	62.1±7.6	56.7±10.2

TABLE S5

Comparison of agricultural characters between Wuyujing 3 and Wuyunjing 8 background NILs. Data are means±s.d. (n=10-20)

Traits	Wuyujing 3 (*qpe9-1*)	Wuyujing 3 (*qPE9-1*)	Wuyunjing 8 (*qpe9-1*)	Wuyunjing 8 (*qPE9-1*)
Plant height (cm)	78.9±1.9	102.5±2.2	95.6±5.5	113.8±12.9
Panicle length (cm)	15.8±0.8	19.2±2.5	16.9±0.6	20.1±1.5
Grain length (mm)	7.3±0.2	8.1±0.2	7.4±0.2	8.3±0.2
Grain width (mm)	3.2±0.2	3.2±0.1	3.3±0.1	3.2±0.3
Grain thickness (mm)	2.1±0.1	2.1±0.1	2.2±0.1	2.2±0.1
1,000 grain-weight (g)	26.8±1.1	29.3±1.4	27.8±0.9	31.3±1.1
Grain yield per plant (g)	22.5±3.2	26.3±5.5	23.3±4.8	26.9±4.9
Internode length (cm)				
-1	21.9±1.7	33.5±2.6	25.8±1.6	30.1±3.7
-2	17.5±0.5	23.7±0.6	20.1±1.2	25.6±3.6
-3	12.8±0.7	16.5±1.8	15.3±0.9	20.9±5.1
Leaf length (cm)				
-1	16.3±2.0	29.0±3.9	20.7±4.9	27.8±6.1
-2	28.9±4.4	44.2±4.3	36.4±3.2	40.9±5.3
-3	32.9±0.9	45.8±0.8	38.2±4.4	47.5±2.7
Leaf width (cm)				
-1	1.8±0.2	1.7±0.2	1.6±0.2	1.6±0.2
-2	1.4±0.1	1.4±0.1	1.4±0.1	1.3±0.1
-3	1.2±0.1	1.1±0.1	1.3±0.1	1.2±0.1
No. Spikelets on the main panicle	112.5±8.3	118.0±15.2	142.6±20.5	159±15.4
No. primary branch	11.3±0.9	10.2±1.5	12.3±0.5	11.6±0.8
No. secondary branch	25.2±1.2	26.3±2.6	28.3±1.6	29.2±2.2