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ERECT PANICLE2 Encodes a Novel Protein That Regulates Panicle Erectness in *Indica* Rice

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ABSTRACT

Rice (*Oryza sativa* L.) inflorescence (panicle) architecture is an important agronomic trait for rice breeding. A number of high-yielding *japonica* rice strains, characterized by an erect panicle (EP) of their architecture, have been released as commercial varieties in China. But no EP-type *indica* varieties are released so far. Here, we identified two allelic erect-panicle mutants in *indica* rice, ***e****rect* ***p****anicle2-1* (*ep2-1*) and *erect panicle2-2* (*ep2-2*), exhibiting the characteristic erect panicle phenotype. Both mutants were derived from spontaneous mutation. We cloned the *EP2* gene by way of a map-based cloning strategy, and a transgenic complementation test rescued the phenotype of *ep2-1*. Anatomical investigations revealed that the *ep2* mutants have more vascular bundles and a thicker stem than that of wild-type plants, explaining the panicle erectness phenotype in *ep2* mutants. It was shown that *EP2* was specifically expressed in the vascular bundles of internodes by GUS staining and RT–PCR. *EP2* encodes a novel plant-specific protein, which localizes to the endoplasmic reticulum with unknown biochemical function. In addition, *EP2* also regulates other panicle characteristics, such as panicle length and grain size, but grain number per panicle shows little change, indicating that the mutation of the *ep2* gene could be applied in EP-type *indica* rice breeding.

FOOD shortage is one of the most serious global problems. The United Nations Food and Agricultural Organization (FAO) estimates that more than 963 million people worldwide were undernourished between 2007 and 2008 (http://www.fao.org/docrep/011/i0291c/i0291c00.htm). The food shortage will be more serious due to continued population growth and increasing competition for arable land between food and energy crops. As one of the most important cereal crops, rice (*Oryza sativa* L.) is widely cultivated worldwide, providing a carbohydrate source for more than half of the world's population. Rice panicle architecture, which determines grain yield, is one of the most important agronomical traits. As such, the diversity of rice panicle architecture has long been an interest of many breeders.

Rice panicle architecture is determined fundamentally by the number of primary branches (length of main axis) and the number of spikelets on each primary branch (length of the primary branch) (Ikeda *et al.* 2005). Unlike Arabidopsis, which produces floral meristems directly from the inflorescence meristem with an indeterminate growth habit, rice has an inflorescence that generates branches and spikelet meristems before producing floral meristems in a determinate pattern (Wang and Li 2005). Several mutants affecting the panicle architecture have been reported in rice, but only a few genes have been isolated. In the *frizzy panicle* (*fzp*) mutant, the formation of florets is replaced by sequential rounds of branching (Komatsu *et al.* 2001, 2003b). The *FZP* gene, encoding an ethylene-responsive element-binding factor (ERF), is homologous to maize *BRANCHED SILKLESS 1* (*BD1*) and *BD1B* (Chuck *et al.* 2002). The *lax panicle* (*lax*) mutant is defective in panicle lateral meristem initiation and has a strongly reduced number of primary branches and spikelets. *LAX* encodes a transcription factor that contains a basic helix-loop-helix (bHLH) domain and is expressed in the boundary between the shoot apical meristem and the region where new meristem forms (Komatsu *et al.* 2001, 2003a). The *aberrant panicle organization1* (*apo1*) mutant exhibits an abnormal panicle phyllotaxy (distichous phyllotaxy) and a decrease in the number of primary branches (Ikeda *et al.* 2005, 2007). *APO1* is an ortholog of the Arabidopsis *UNUSUAL FLORAL ORGAN*

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession no. GQ449684.

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(*UFO*), which encodes an F-box protein that regulates meristem fate and plays a role in preventing the inflorescence meristems from converting precociously into spikelet meristems (Samach *et al.* 1999). *Gn1a*, which controls grain productivity in rice, encodes cytokinin oxidase/dehydrogenase (OsCKX2). Reduced expression of *Gn1a* causes cytokinin accumulation in inforescence meristems and increases the number of reproductive organs, resulting in enhanced grain yield (Ashikari *et al.* 2005). *LONELY GUY* (*LOG*), which encodes a novel cytokinin-activating enzyme, converts inactive cytokinin nucleotides into their active free-base forms. A loss-of-function mutation in *LOG* gives rise to small panicles with fewer branches and less severe defects in the flower organs (Kurakawa *et al.* 2007). *SHORT PANICLE1* (*SP1*) encodes a putative transporter that belongs to the peptide transporter (PTR) family and its mutation causes a defect in rice panicle elongation (Li *et al.* 2009). Most of these studies are concerned with the number of spikelets or panicle size but not the erect panicle (EP), although the EP phenotype is an important agronomic trait.

The first-reported EP variety, Guihuahuang, was identified by the Taihu Institute of Agricultural Sciences in China in the 1960s. Since the 1980s, a great number of EP rice varieties, such as Liaojing 5, have been developed in northern China, leading to a dramatic increase in crop yield (Chen *et al.* 2007). These varieties exhibit a higher yield potential than other common *japonica* varieties due to their higher photosynthetic capacity (Zhang *et al.* 2002). Although the EP trait has been extensively propagated, its mode of inheritance has not been fully elucidated. It was first reported that EP is controlled by a recessive gene, but others reported that EP is controlled by a major gene with dominant or additive effects and modified by polygenes (Zhang *et al.* 2002). Recently, a QTL responsible for the erect panicle trait, *DEP1*, was cloned on chromosome 9 (Yan *et al.* 2007; Huang *et al.* 2009; Zhou *et al.* 2009). The *DEP1* mutant, derived from a gain-of-function mutation caused by the truncation of *DEP1*, has enhanced meristematic activity, resulting in a reduced length of the inflorescence internode and an increased number of grains per panicle. To date, all EP varieties are derived from *japonica* varieties; no EP-type *indica* varieties have been used. Thus, the genes that control the erect panicle are still unknown in *indica* rice.

To deepen our understanding of the processes controlling panicle architecture, especially in *indica* rice, we identified and cloned a new erect panicle gene, ***E**RECT **P**ANICLE 2* (*EP2*), on chromosome 7 by map-based cloning. A loss-of-function mutation in *EP2* leads to the erect panicle phenotype. Cloning of the *EP2* locus showed that it encodes a plant novel protein of unknown biochemical function. The *EP2* gene is mainly expressed in vascular bundles, and the EP2 protein is localized to the endoplasmic reticulum.

MATERIALS AND METHODS

Plant materials: We identified two single recessive rice (*O. sativa L.*) mutants that showed erect panicle. These two were allelic and designated *erect panicle2-1* (*ep2-1*) and *ep2-2*. Both mutants were derived from spontaneous mutation. *ep2-1* was isolated from the *indica* cultivar Zhongxian 3037 and *ep2-2* was collected from the *indica* cultivar 93-11. These two mutants were derived from different genetic backgrounds; thus, we used the original cultivar of each mutant as a control.

The F_2 mapping population was generated from a cross between *ep2-1* and Zhonghua 11, a polymorphic *japonica* cultivar. The self-fertilized heterozygous plants in the F_2 population constructed a large F_3 population for fine mapping. All the rice plants were grown in the paddy fields under normal growth conditions.

DNA extraction and positional cloning: Genomic DNA was extracted from rice leaves using the CTAB method. For fine mapping, PCR-based markers were developed on the basis of sequence differences in the *japonica* variety Nipponbare and *indica* variety 93-11 (http://www.gramene.org/resources/). The primer sequences of the molecular markers that were used are listed in supporting information, Table S1. PCR products were separated by electrophoresis on 3.0% (w/v) agarose gels; then, linkage analysis was performed between the *ep2* mutation and STS markers.

Complementation test: An 11.48-kb genomic DNA fragment containing the entire *EP2* coding region, the 2797-bp upstream sequence, and the 1584-bp downstream sequence was inserted into the binary vector pCAMBIA1300 to generate the transformation plasmid pCEP for complementation tests. A control plasmid, pCEPS, containing the truncated EP gene was also constructed. The two binary plasmids were introduced into *Agrobacterium tumefaciens* EHA105 by electroporation and transformed into rice for complementation testing according to a published method (Hiei *et al.* 1994).

Histological analysis: For scanning electron microscopy (SEM), samples at the mature stage were fixed overnight at 4° in formalin:glacial acetic acid:70% ethanol (FAA) at 1:1:18. After dehydration in a graded ethanol series and substitution with isoamyl acetate, the samples were critical-point dried, sputter coated with gold, and observed under a scanning electron microscope (S-4000; Hitachi, Tokyo) at an accelerating voltage of 10 kV.

Gene expression analysis: Total RNA was extracted from various tissues (roots, internodes, leaf blades, and panicles) of Nipponbare at the heading stage, using a TRIpure reagent (BioTeke) as described by the supplier. For semiquantitative RT–PCR analysis, total RNA (3.5 μg) was treated with RNase-free DNase, and first-strand cDNA was synthesized through reverse transcription by an oligo(dT) primer (TaKaRa). Subsequently, the first-strand cDNA was used for PCR amplifications with the following gene-specific primer pairs: 5′-TGG AAGGGGCTAGGAAGATT-3′ and 5′-CACAAGCCGTGACAA GGTC-3′ for *EP2*, 5′-TACTTCTGGGACACCCTGCTC-3′ and 5′-ACTCCCCGTGCGGATCAAAC- 3′ for *OsCRY1*, 5′-AGTAC CCTTGAGGCTCTTCTGC-3′ and 5′-ATACAGCAGATCACCA TTAAAACTC-3′ for *OsCRY2*, 5′-TTTGTCTCGTCTGCCTCC TCC-3′ and 5′-ACATCTTCTGCCTTGATTACCTG-3′ for *OsPHYA*, 5′-TGATTCCTCGGTGGAGTGTAT-3′ and 5′-AAACCT GGACAGCAAACACC-3′ for *OsELF3*, 5′-AGAAAGCAAAAGT TGGAAGAGC-3′ and 5′-ATCCGAGCAAGTGAGAACATC-3′ for *OsCOP1*, and 5′-CAAGATGATCTGCCGCAAATGC-3′ and 5′-TTTAACCAGTCCATGAACCCG-3′ for *UBQ*. The PCR samples were collected after 30 cycles for *EP2*, *OsCRY1*, *OsCRY2*, *OsPHYA*, *OsELF3*, and *OsCOP1* or 25 cycles for *UBQ*.

For the promoter–GUS assay, a 3237-bp genomic fragment upstream of the *EP2* gene translation start codon was PCR

amplified with the primers 5′-GAATTCCAGGCTGACTT GACAGAGG-3′ and 5′-CCATGGGGCGAGGTCGGATCTGG TGG-3′. The DNA fragment was cloned into the *Eco*RI and *Nco*I sites of the vector pCAMBIA1301. The resulting plasmid was transformed into rice, and the transgenic plants were analyzed with a GUS staining assay as previously described (Hagen *et al.* 1991).

Phylogenetic analysis: The BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/) was used to look for protein sequences homologous to EP2 protein. The alignment of amino acid sequences was carried out by using ClustalX 2.0 (Jeanmougin *et al.* 1998), and a neighbor-joining tree was generated by using MEGA version 2.1 (Kumar *et al.* 2001).

Subcellular localization of the GFP-EP2 fusion protein: The *EP2-GFP* fusion gene was constructed by fusing the *GFP* open reading frame (ORF) to the C-terminal end of the *EP2* ORF (amplified from AK120310 with primers 5′-AAGCTTA TGGAGCCCGACGCCCCG-3′ and 5′-GGATCCCCTGAGCCT TGCATCACCCC-3′) and cloning into the vector pJIT163. The EP2-GFP construct and the endoplasmic reticulum (ER)-mCherry were cotransfected into rice protoplasts, using a polyethylene glycol-calcium-mediated method followed by an 18-hr incubation to allow transient expression. The transformed protoplasts were examined using a Leica TCS SP5 confocal system.

RESULTS

Characterization of *ep2* mutants: The erect panicle of rice is one of the most important agronomic traits contributing to grain yield. We identified two rice erect panicle mutants, *ep2-1* and *ep2-2*, from the *indica* cultivars Zhongxian 3037 and 93-11, respectively. Phenotypic analysis indicated that the morphology of *ep2* mutants is similar to that of wild-type plants from the vegetative developmental stage to the early reproductive stage; however, after grain filling, the *ep2* mutant shows a slight reduction in plant height (Figure 1, A and B; Table S2). The *ep2* mutant exhibits a remarkable phenotype in panicle architecture. The panicle of the *ep2* mutants keeps erect from flowering to seed maturity, while the panicle of the wild-type plant starts to droop after flowering (Figure 1, C and D). Compared to the wild type, the *ep2-1* mutant truncates 50% of the panicle length, whereas the *ep2-2* mutant has an 18.1% truncation in panicle length (Table S2). Although the grain number per panicle is higher in the mutant, no significant difference was found in the number of primary and secondary branches between the *ep2-2* mutant and wild type (Table S2). The *ep2-1* mutant, however, has fewer secondary branches, which results in a decrease of grain number per panicle. In addition, the grain size is different in both mutants (Figure 1, E and F). We observed a substantial decrease in the *ep2-1* grain length but the grain width and grain thickness were increased (Table S2). In the *ep2-1* mutant, the 1000-grain weight was found to be 20.7 g, compared to 24.6 g in Zhongxian 3037. On the other hand, the 1000-grain weight was found to be decreased only slightly in the *ep2-2* mutant, 29.6 g in 93-11 and 28.7 g in *ep2-2* (Table S2).

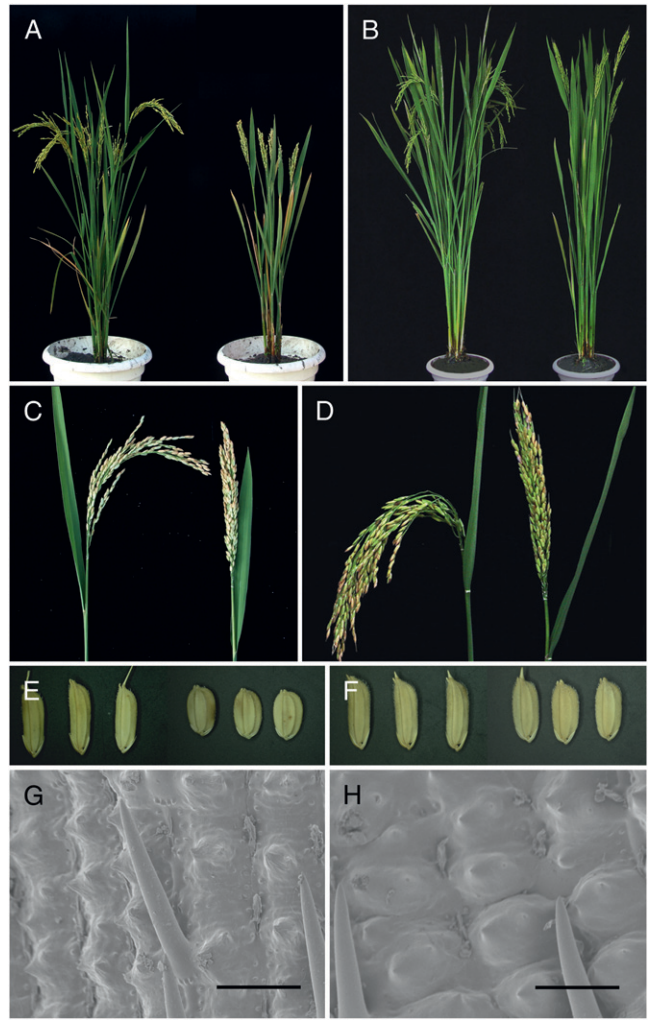

Figure 1.—Phenotype of the *ep2* mutants. (A) Phenotypic comparison between Zhongxian 3037 (left) and *ep2-1* (right) after heading. (B) Phenotypic comparison between 93-11 (left) and *ep2-2* (right) after heading. (C) Comparison of panicle size and architecture between Zhongxian 3037 (left) and *ep2-1* (right). (D) Comparison of panicle size and architecture between 93-11 (left) and *ep2-2* (right). (E) Comparison of mature grains between Zhongxian 3037 (left) and *ep2-1* (right). (F) Comparison of mature grains between 93-11 (left) and *ep2-2* (right). (G and H) Scanning electron microscopy (SEM) shows the outer surface of rice glumes of Zhongxian 3037 (G) and *ep2-1* (H). Bars, 100 μm.

To understand whether the difference in grain size was caused by a decrease in cell number and/or cell size, we used SEM to observe the epidermis cells outside of the rice glumes. SEM showed that the epidermis cells outside of the rice glumes of *ep2-1* are shorter but wider than those of wild type (Figure 1, G and H), leading to changes in grain size.

The *ep2* mutant has more small vascular bundles in the uppermost internodes: In rice, the vascular bundle systems of the culms and leaf veins provide mechanical support to the plant (Ye 2002; Li *et al.* 2003; Teale *et al.* 2006). The erect panicle of *ep2* mutants indicates that the panicle of the mutant is mechanically stronger than

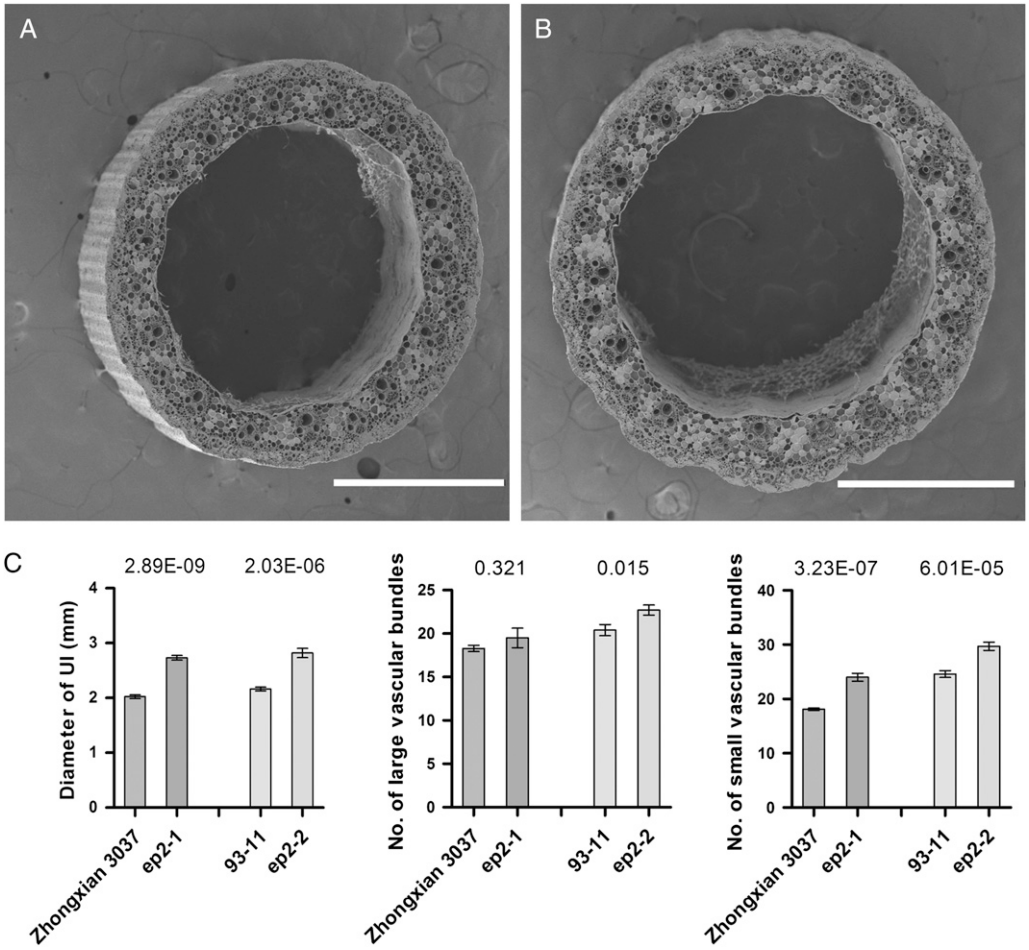

FIGURE 2.—Histological analyses of uppermost internodes (UIs) at the mature stage. (A and B) SEM shows the differences of UIs transverse sections between Zhongxian 3037 (A) and *ep2-1* (B). Bars, 1 mm. (C) Comparison of the diameter, large vascular bundle, and small vascular bundle of UIs. Data are mean ± SD ($n = 20$). A Student's *t*-test was used to generate the *P*-values.

that of the wild-type plant. Therefore, we examined the morphological phenotype of the uppermost internodes (UIs) in the *ep2-1* mutant and the wild-type plant. SEM observation revealed that there was a remarkable difference in the diameter of UIs between the *ep2-1* mutant and wild type at the mature stages (Figure 2, A and B). The diameter of the UIs in the *ep2* mutant was found to be larger, nearly 3 mm compared to ~2 mm in wild type. A further comparison of the mature UIs revealed that the *ep2* mutant has more vascular bundles than the wild-type plant, especially small vascular bundles (Figure 2C). These results suggest that the *ep2* mutant has a thicker stem and more vascular bundles, which increases the mechanical strength of the internode and results in the erectness of the panicle.

Identification of the *EP2* gene: The *ep2-1* mutant was isolated from an *indica* cultivar Zhongxian 3037. For genetic analysis of the *ep2-1* mutant, an F_2 population was generated from the cross between the *ep2-1* mutant and Zhonghua 11. All the F_1 plants showed wild-type phenotype, while the F_2 plants segregated into two groups: 597 plants showed wild-type whereas 224 plants showed erect panicle phenotype similar to that of the *ep2-1* mutant in a 3:1 ratio ($\chi^2 = 2.16$; $P > 0.05$). This result showed that *ep2* is a single recessive nuclear gene.

To clone the *ep2* gene through a map-based cloning strategy, we used a mapping population derived from crosses between *ep2-1* and Zhonghua 11 (*japonica*). A total of 224 segregates with the *ep2-1* mutant phenotype were used for primary gene mapping. Linkage analysis showed that the *EP2* locus is located between the molecular markers P1 and S5865 on the long arm of chromosome 7 (Figure 3A). To further map the *EP2* locus, a large F_3 population derived from the original F_2 population was used. We then fine mapped the locus to a 30.3-kb region between markers P5 and P7, using newly developed molecular markers (Figure 3B and Table S1). Database (KIKUCHI *et al.* 2003) searches revealed three ORFs, which were confirmed by full-length cDNA clones in this region (Figure 3B). By sequencing PCR-amplified genomic fragments, one candidate gene, *Os07g42410*, was found to be mutated in both *ep2* alleles (Figure 3C). The *ep2-1* allele has a single G to T nucleotide substitution (2383G > 2383T) in the seventh exon; this mutation substitutes Val (GAA) with a stop codon (TAA) and leads to the premature termination of the protein. The *ep2-2* allele has a single-nucleotide deletion, resulting in a frameshift mutation in the ORF (Figure S1). These results suggest that the gene *Os07g42410* controls the phenotype of mutants.

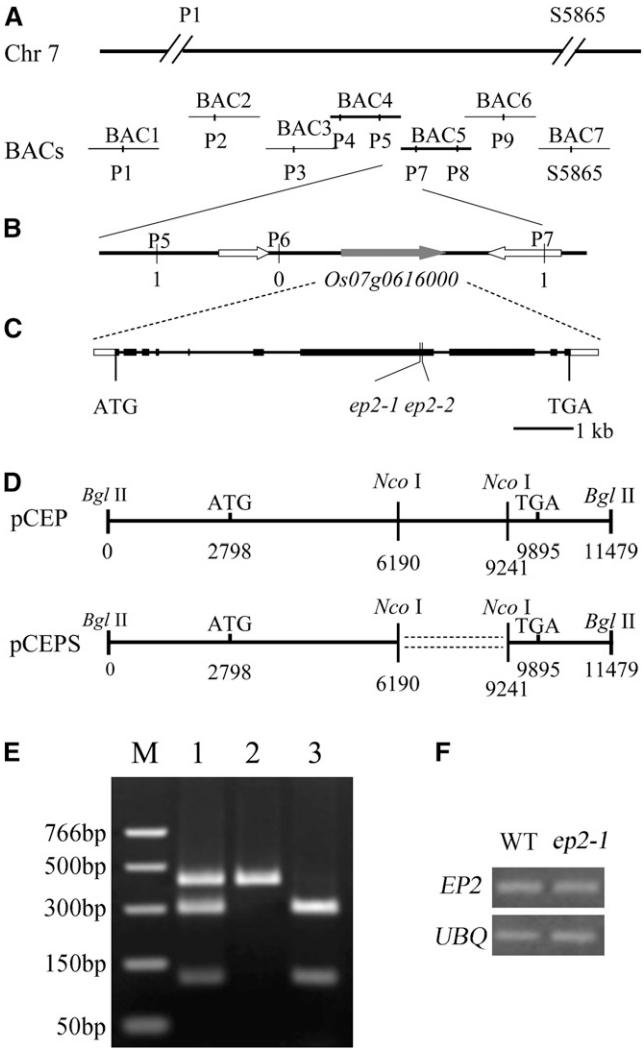

FIGURE 3.—Cloning and confirmation of the *EP2* gene. (A) The *EP2* locus was mapped to a region between markers P1 and S5865 on the long arm of rice chromosome 7 (Chr7). (B) Fine mapping of the *EP2* locus using primers developed from seven bacterial artificial chromosomes (BACs): BAC1, AP006458; BAC2, AP005895; BAC3, AP003700; BAC4, AP005198; BAC5, AP004988; BAC6, AP004309; and BAC7, AP004380. P1–P9 are the developed markers in this study (Table S1). (C) *EP2* gene structure, showing the mutated sites of the two *ep2* alleles. The start codon (ATG) and the stop codon (TGA) are indicated. Solid boxes indicate the coding sequence, open boxes indicate the 5′- and 3′-untranslated regions, and lines between boxes indicate introns. Mutant sites in *ep2-1* and *ep2-2* are also shown. (D) Complementation constructs. The construct pCEP contains the entire *EP2* gene, plus a 2797-bp upstream region and a 1584-bp downstream region. The plasmid pCEPS contains a partial coding region of the ORF. (E) Identification of transgenic plants. The G to T substitution in *ep2-1* disrupts the *Dde*I site that was used for identifying the wild-type line. Lane M, PCR marker; lane 1, the pCEP-transformed rice line; lane 2, *ep2-1*; lane 3, wild type. (F) Comparison of *EP2* transcript levels in 6-cm panicles between the wild-type and *ep2-1* plants by RT–PCR.

The identity of *EP2* was further confirmed by genetic complementation experiments. The plasmid pCEP, containing the entire *Os07g42410* ORF, and pCEPS, containing a partial coding region of the ORF (Figure 3D), were independently introduced into recessive mutants of the F_3 generation of the mapping population. All 18 transgenic lines of pCEP showed complementation of the *ep2-1* phenotype, whereas none of the 9 lines containing pCEPS rescued the *ep2-1* mutant phenotype. The mutation in *ep2-1* disrupts the *Dde*I site in the genomic DNA; this change was used as a CAPS marker to identify the *ep2-1* mutant background in the complementation test (Figure 3E). Thus, we cloned the *EP2* gene, which controls the *ep2-1* phenotype in rice. RT–PCR analysis showed that the transcription level of the *EP2* gene is not altered in the *ep2-1* mutant (Figure 3F). It seems that the *EP2* transcript is transcribed in the *ep2-1* mutant.

***EP2* encodes a plant-specific protein of unknown biochemical function:** By searching the KOME cDNA database (http://cdna01.dna.affrc.go.jp/cDNA), we identified a full-length cDNA clone corresponding to the *EP2* gene, AK120310 (Rice Genome Research Center of the National Institute of Agrobiological Sciences). Sequence comparison between the genomic DNA and cDNA revealed that the *ep2* gene is composed of 10 exons and nine introns. And it encodes a protein of 1365 amino acids (Figure 3C). Comparing the EP2 protein sequences between Nipponbare (*japonica*) and Zhongxian 3037 (*indica*), four substitutions have been found on the EP2 protein sequences (Figure S1).

A search of the National Center for Biotechnology Information (NCBI) database and the Institute for Genomic Research (TIGR) Gene Indices database identified one *EP2* homolog in *Sorghum bicolor*, one in *Zea mays*, one in *Vitis vinifera*, one in *Populus trichocarpa*, and five in *Arabidopsis thaliana* (Figure 4A); we did not find any other *EP2* homologs in rice. Notably, we identified COP1-interacting protein 7 (CIP7, AT4G27430) and two COP1-interacting protein-related proteins, AT1G17360 and AT1G72410, which share ~39% amino acid sequence identity in the 5′ region (~1–200 aa) (Figure S2). However, *EP2* and *CIP7* belong to two divergent clades, indicating that they likely have distinct functions (Figure 4A). We also compared the expression of some *COP1*-related genes in the wild type and the *ep2-1* mutant and found that their transcription levels are not obviously different in the *ep2-1* mutant (Figure 4B). Taken together, the *EP2* sequence does not match any protein of known biochemical function in the public databases.

Expression pattern of *EP2*: To better understand the function of *EP2*, we examined the expression pattern of *EP2* in various organs, including the roots, internodes, leaf blades, and panicles of Nipponbare at the heading stage. We performed semiquantitative RT–PCR analysis to estimate the level of the *EP2* transcript. As shown in

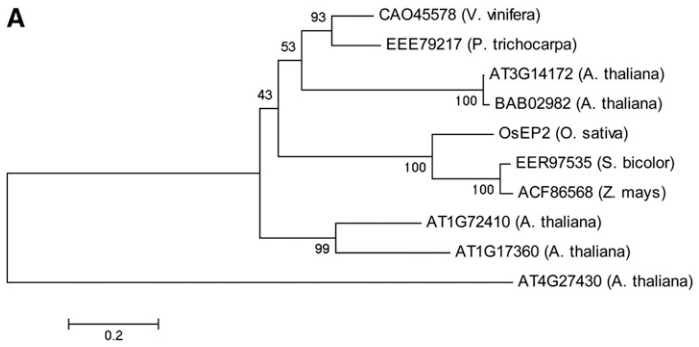

FIGURE 4.—Phylogeny of the EP2 protein family. (A) The amino acid sequences of the entire proteins were aligned by CLUSTALX, and the phylogenetic tree was constructed using the neighbor-joining algorithm. Bootstrap values are shown at each node. The bar is an indicator of genetic distance based on branch length. (B) Semiquantitative RT–PCR analyses of rice *COP1*-related genes between the wild type (WT) and *ep2-1*.

Figure 5A, *EP2* is mainly expressed in the internodes and panicles. We could detect the expression of the *EP2* gene in the leaf blades but not in the roots. In the *ep2-1* mutant, *EP2* has a similar expression pattern (Figure S3). These results show that the *EP2* gene is temporally and spatially expressed.

To assess the developmental regulation of *EP2* expression, we generated transgenic rice plants expressing the 3237-bp fragment of the *EP2* promoter fused to the *GUS* reporter gene. We detected GUS expression in the stem base, axillary buds, lamina joint, and panicle (Figure 5, E–H). We further observed strong GUS activity in the vascular bundles of the internode, leaf sheath, and leaf (Figure 5, B–D). The observation of *EP2* being preferentially expressed in vascular tissues, together with the fact that *ep2* has more small vascular bundles in the internode, implies that *EP2* might control the number of small vascular bundles.

Subcellular localization of EP2: To ascertain the subcellular localization of EP2, *GFP* was fused to the C terminus of *EP2* under the control of the 35S promoter, and the fusion gene was transformed into rice protoplast. Meanwhile, GFP alone was used as the control. As expected, GFP alone was detected both in the cytoplasm and in the nucleus: its small size (26 kDa) allows passive diffusion throughout nuclear pores (Figure 6, A and B). We found that EP2-GFP has a reticulate fluorescence pattern (Figure 6, C and D), suggesting that EP2 may be targeted to the endoplasmic reticulum. To test this idea, EP2-GFP and the ER-specific marker (ER-mCherry) were cotransformed into rice protoplasts. We found that EP2-GFP and ER-mCherry are colocalized (Figure 6, E–H). Therefore, we concluded that EP2 is an endoplasmic reticulum-localized protein.

DISCUSSION

The ideal plant architecture, which is the specific combination of morphological traits that are favorable for plant photosynthesis, growth, and grain yield, has been defined as the crop ideotype (DONALD 1968). In monocotyledonous crops such as rice and maize, the seed productivity is closely allied with inflorescence architecture that provides the primary food source for humans and livestock. Therefore, understanding the molecular mechanisms of inflorescence architecture is practically important in modifying the architecture of inflorescences to breed desirable crops in breeding programs.

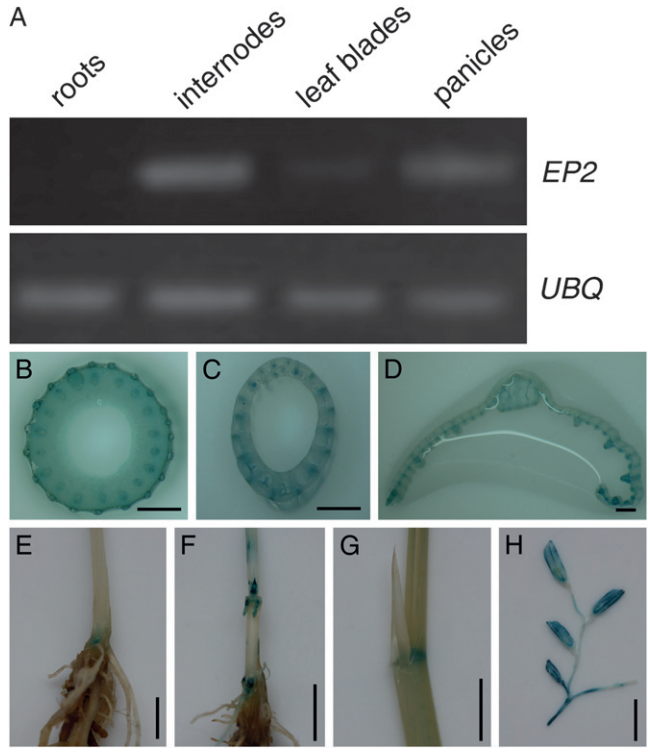

FIGURE 5.—Expression pattern analysis of *EP2*. (A) RT–PCR estimates the relative expression of *EP2*. Total RNA was isolated from roots, internodes, leaf blades, and panicles of Nipponbare at the heading stage. Amplification of the rice *UBQ* gene was used as a control. (B–H) The *EP2* expression was revealed by GUS staining in EP2 promoter–GUS transgenic rice plants. Transverse sections of internode (B), leaf sheath (C), and leaf blade (D) at the heading stage show the expression of EP2 promoter–GUS in the vascular bundles. The expression of *EP2* is also detected in the tiller base (E), the axillary bud (F), the lamina joint (G), and the panicle (H). Bars: B–D, 0.5 mm; E–H, 5 mm.

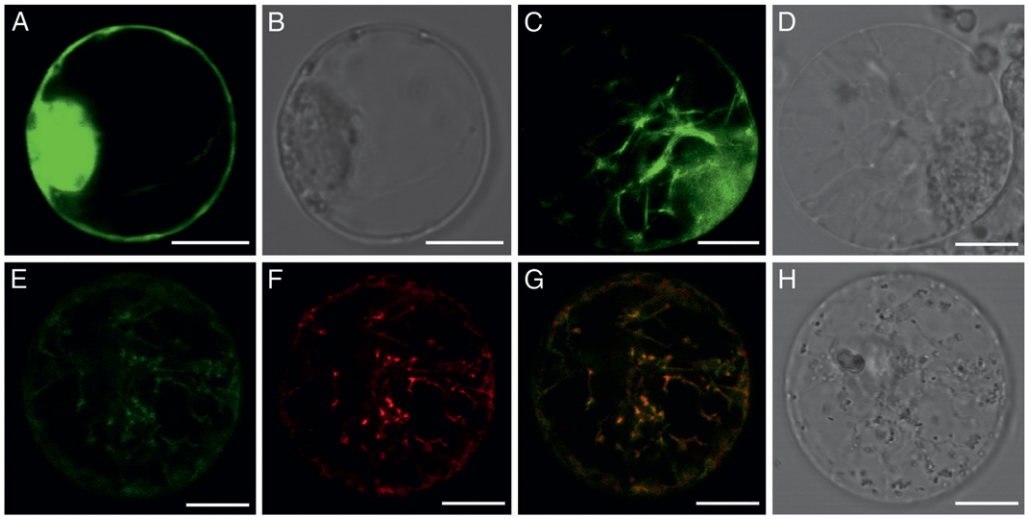

FIGURE 6.—Subcellular localization of EP2-GFP in rice protoplast cells. (A and B) A rice cell expressing GFP alone (A) and its DIC image (B), showing fluorescent signals in nucleus, membrane, and cytoplasm. (C and D) A rice cell expressing EP2-GFP alone (C) and its DIC image (D), showing a reticulate signal pattern. (E–H) A rice cell expressing EP2-GFP (E), the endoplasmic reticulum marker ER-mCherry (F), a merged image (G), and the DIC image (H). Bars: A and B, 7.5 μm; C–H, 10 μm.

The EP-type rice has higher photosynthetic capacity that leads to high yields in *japonica* varieties (CHEN *et al.* 2007; HUANG *et al.* 2009). At present, the EP-type rice has been widely cultivated in most of the *japonica* rice growing regions in China (YAN *et al.* 2007). While there are large numbers of high-yield EP varieties that are currently being widely cultivated, the inheritance of the EP phenotype has not been fully elucidated. Recently, two groups cloned the *EP* gene that applied in *japonica* varieties to chromosome 9 (HUANG *et al.* 2009; ZHOU *et al.* 2009). However, there are no EP-type *indica* varieties available. In this study, we identified two new EP mutants, *ep2-1* and *ep2-2*, from different *indica* varieties and mapped the gene on chromosome 7. The panicle of *ep2* mutants keeps erectness from flowering to seed maturity. Furthermore, the *ep2* mutants have thicker stems and more vascular bundles than wild-type plants, which is favorable for both stem mechanical strength and water transport capacity. These characteristics are important factors for the breeding of high-yielding and lodging-resistant varieties. Although the 1000-grain weight of the *ep2* mutants was slightly less than that of wild plants, the grain number per panicle was increased in the *ep2-2* mutant (Table S2). Additionally, the EP-type rice improves plant architecture and population quality, which increases the grain production (ZHOU *et al.* 2009). Taken together, our results indicate that *ep2* is a useful allele for increasing grain yield in *indica* rice.

In Arabidopsis, COP1 acts as an E3 ubiquitin ligase to repress light signaling by targeting photoreceptors and downstream transcription factors for ubiquitylation and degradation (YI and DENG 2005). Homology searches indicated that EP2 is similar to the COP1-interacting protein-related protein in the 5′ region (Figure S1). However, no changes in the expression of *COP1* and its related genes were found between the *ep2-1* mutant and wild-type plants. The relation between EP2 and COP1 is still to be identified at the protein level.

Examination of the panicles revealed that the number of small vascular bundles in the uppermost internodes of *ep2* is more than that in wild type. SEM showed that the epidermis cells outside of the rice glumes in *ep2-1* are shorter but wider than those in the wild type, which might be responsible for the alteration of grain shape. Hormones play important roles in pattern formation of the vascular system (SCARPELLA *et al.* 2003). In addition, it has been shown that gibberellin treatment increases both large and small vascular bundles of the internodes (SHIMIZU and TAKEOKA 1966). Considering the similarities between *ep2* mutant and gibberellin-treated plants, it is likely that *EP2* might be involved in gibberellin signaling that is required for the normal development of panicles. The detailed molecular mechanism still needs to be explored in future studies.

In summary, we described two new EP mutants in this study. Characterization of *EP2* provides new insights into the morphological establishment of rice panicle architecture. The *ep2* mutants may provide a useful germplasm resource for developing new high-yielding EP-type *indica* varieties. Further investigation of *EP2* would facilitate our understanding of the molecular regulatory mechanism of rice panicle architecture development.

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ERECT PANICLE2 Encodes a Novel Protein That Regulates Panicle Erectness in *Indica* Rice

Keming Zhu, Ding Tang, Changjie Yan, Zhengchang Chi, Hengxiu Yu, Jianmin Chen, Jiansheng Liang, Minghong Gu and Zhukuan Cheng

DOI: 10.1534/genetics.109.112045

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EP2(J)  MEPDAPLDFALFQLSPRRSRCELVVSGNGRTERIASGSVKPFVAHLRAAEEQAAAQPPPPAIRLQLDRRAAWFSKGTLER
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  FVRFVSTPEVLEMANTFDAEMSQLEGARKIYAQGVAGGADGAESAAAADITKKELLRAIDVRLSALKQDLVTACARASSA
EP2(I)  S+++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  GFNPDSVSELVLFADHFGANRLSEACNKFMSLCQRRPDICPHYSVSSTSSQWKSFDDGNVRGSSSSDMSLDETQADQGAS
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  SNKSIIGGSVSHIHRSNSQNSVDVPPEPSAVQHPKPTIQQSVEKQEKETDALPAPAPAGGGSRRLSVQDRINMFESKQKE
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  QTSSSGNSAACTSKVVPTKGEHRRVPSGASMDKLVRRWSNVSDMSIDLSNNDSSSLNEKREIGTPVGTPTSANLEVNSKA
EP2(I)  +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++N++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  RADGDANGLKHAVTSCQKDTSDALPLDSTTADAFSSSTLNTTSPSPLSAIASSSPQKQTAPRVEDDMVITSSIESESSFR
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  KEVGASQGKGDVRMSGQAVSSVSTRARVKTSPRPTWPENNVTLSSPPLSQEHVQMTDEETIPIVHEVAVKKEQIVQKDNR
EP2(I)  +++++++++++++++++++++++++++++++++++L++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  GSRLRSKEIHAEADVVGRKDRPSRTTGKISDTRTRATSNPRANFRGSSVRDEAASTEAEVHDVNLQRKSLARKVEDSGRK
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  VAAGSEILPQSDCSIHQGTNLSRQSSSAEQELSLHGGKVKLISDGNAVPLEQTKRPTKGSQDRHDELQKKANELEKLFAA
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-2   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

EP2(J)  QKLTSSRRGKSTDVQVENTPRVNEVKPPLVLPERIYTKQIVKESITNEFDANELLKMVDTEGYNNNVPQSIISLEESRGK
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1   ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++*
EP2-2   +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++S

EP2(J)  FYDQYMQKRDAKLKEDWKLQGEQKEATIKAMRDSLERSNAEMRAKFSRSSSVPDSTYISRCAHKFPPLQSVIKDKDQGID
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1
EP2-2   SMINICRRGMQN*

EP2(J)  SFLVEEEMNSDYLSGDGSSRSADSRKHFSNKVACNQKKSIAPVHRHSSRTVSSGYANRRNLPDNPLAQSVPNFADLRKEN
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1
EP2-2

EP2(J)  TKPSAGLSRAAPRTQPKSFIRSKSIIEESKNISKDQSRKSQSMRKNLSPGELRDATSMNDVIYNWAPSKISNDQVEGVFA
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1
EP2-2

EP2(J)  YITHTAGSTKSFLRKGNEAHPAVGIAGFAPPMFANTYQNGDDDDFLDQEEDSPDETKDEEYESIEENLRESDFPADSDSE
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1
EP2-2

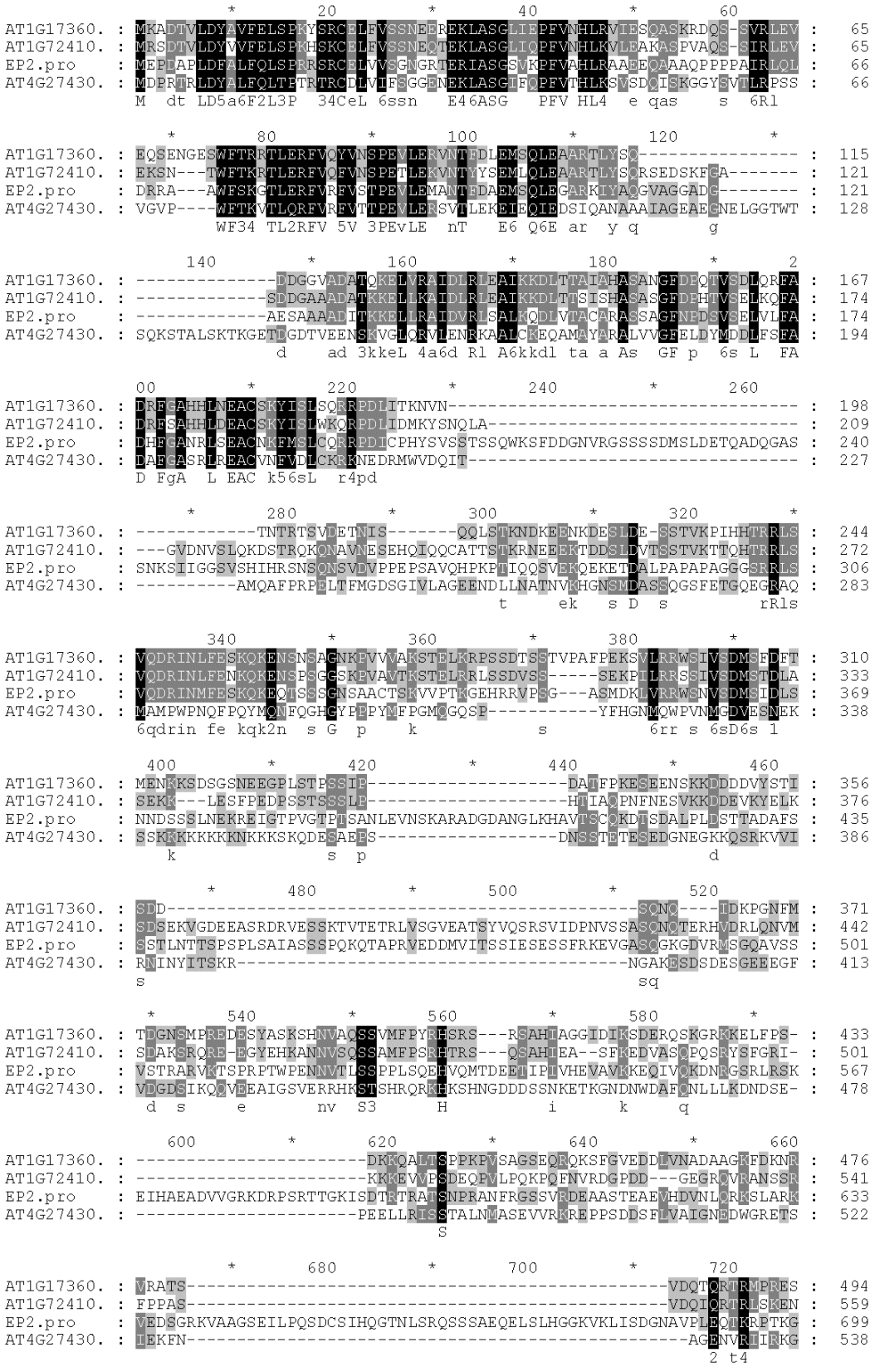
EP2(J)  NPGISHEFGNSDDPGSENGDVSFPSDAPTLGGSKFNAFAGNMHDTPGEVPASWSTRPHLFAYANDNSDGDAFADSPNGSP
EP2(I)  +++++++++++++++++++++++++++++++C++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1
EP2-2

EP2(J)  SPWNSHTLDQITDADVSRMRKKWGSAQMPFVGPNASQQPRKDVTKGFKKLLKFGRKTRGADGLNDWVSASTASECDDDME
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1
EP2-2

EP2(J)  DGRDLAMGSSDDFRKSRMGYPSAYDGFVDTDVFAEQDQSLRSSIPNPPANFRLREDQLTGSSLKAPRSFFSLSTFRSKGG
EP2(I)  ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
EP2-1
EP2-2

EP2(J)  DARLR*
EP2(I)  +++++*
EP2-1
EP2-2
```

FIGURE S1.—Alignment of deduced amino acid sequences of wild-types (*japonica* and *indica*) and mutant *ep2s*. Amino acids identical to those of wild-type (*japonica*) EP2 are shown as +. Amino acids different from those of wild-type (*japonica*) are shown in bold. An asterisk shows a position of the stop codon. J, *japonica*; I, *indica*.



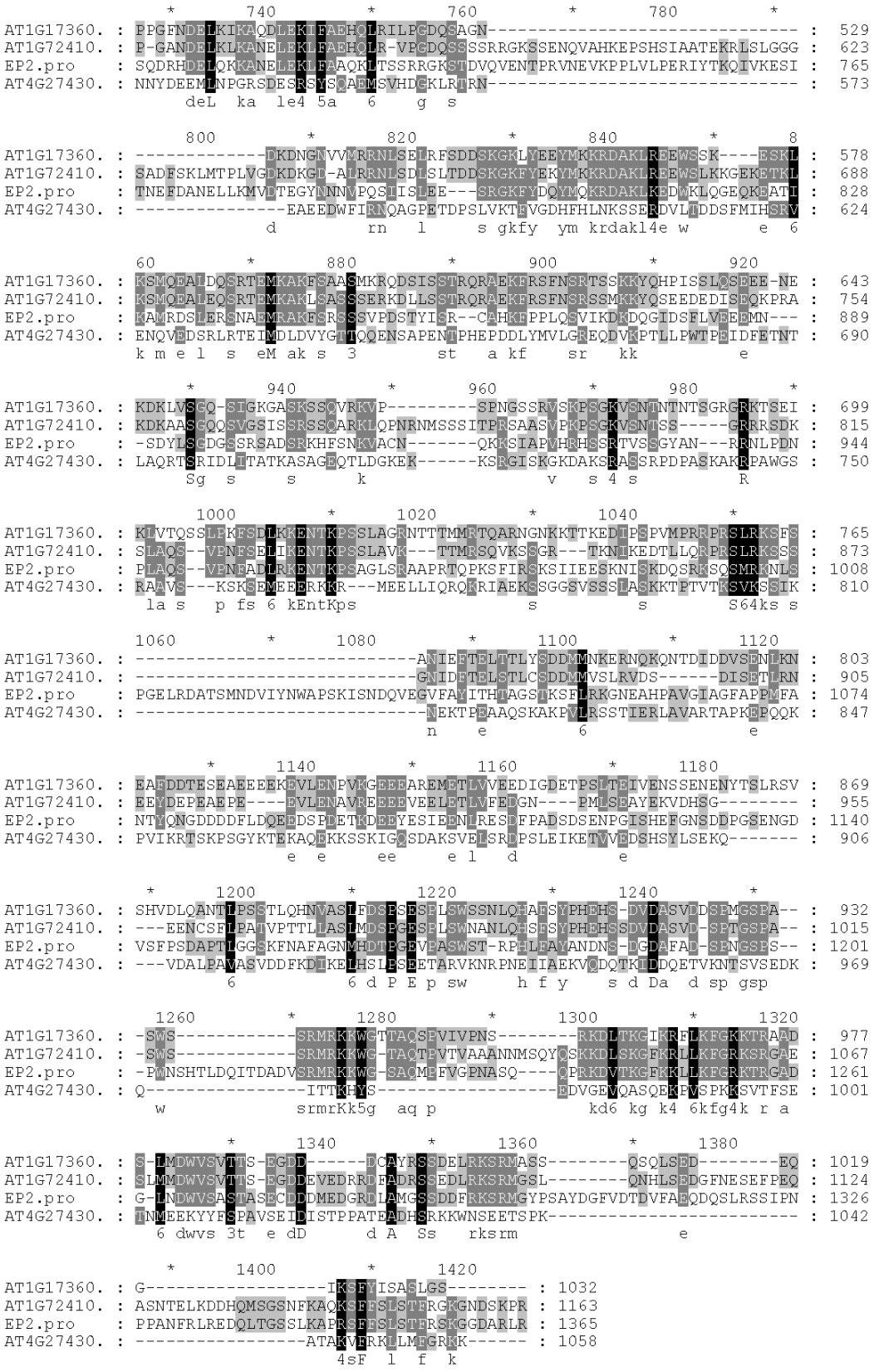


FIGURE S2.—Alignment of the EP2 amino acid sequence with Arabidopsis AT4G27430, AT1G17360, AT1G72410. Identical and conserved residues are indicated by dark gray boxes and variant residues by light gray boxes.

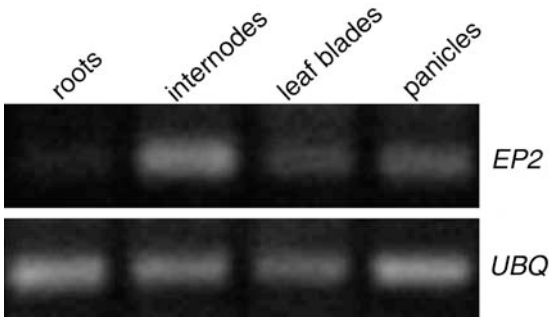


FIGURE S3.—RT-PCR estimates the relative expression of the truncated *EP2*. Total RNA was isolated from roots, internodes, leaf blades and panicles of mutant *ep2-1* at the heading stage. Amplification of the rice *UBQ* gene was used as a control.

TABLE S1

List of the PCR-based molecular markers developed in this study

Marker	Primer Pairs[a]	Fragment Size[b]
P1	F, 5'-AGTTGCCGACAATGTAAGCC-3';	N=257, Z=234
	R, 5'-GGGTATAACGATTCCTCCAACTA-3'	
P2	F, 5'-TCCAAACCCAAATCAACG-3';	N=377, Z=346
	R, 5'-TATCATTAAGCCGTCTTCG-3'	
P3	F, 5'-TCCTTTACCTCTTCCGTTC-3';	N=298, Z=264
	R, 5'-TACGAATATCCTTATCAAGCC-3'	
P4	F, 5'-CAATTTGGCTTTTCCTACCCC-3';	N=246, Z=222
	R, 5'-TCCCACATAACTCGGGCATC-3'	
P5	F, 5'-TAGAGGGCTTTAGGGATATAG-3';	N=115, Z=96
	R, 5'-ACTCGTAATGCTTGTTTGC-3'	
P6	F, 5'-TGCGATCATTTTAACGGTTCT-3';	N=225, Z=212
	R, 5'-ATTAGGCTCAAAAGATTCGTC-3'	
P7	F, 5'-AACCTGGCGACGAGATAACC-3';	N=133, Z=125
	R, 5'-GCAAAGAAGTGGAGATGTGGG-3'	
P8	F, 5'-TAAGCATGGTTACCTGTTCG-3';	N=263, Z=240
	R, 5'-TATTCTATGGGCTTGTTTGG-3'	
P9	F, 5'-GATGGAGGCAACCAAAACC-3';	N=169, Z=147
	R, 5'-TGGACCCTTGTCGCTGAAC-3'	

[a] F, forward primer; R, reverse primer.
[b] Number indicate the size (in bp) of amplified fragments. N, Nipponbare; Z, 93-11.

TABLE S2

Morphometric analysis of wild-type and *ep2* mutants

	Zhongxian 3037	*ep2-1*	93-11	*ep2-2*
Plant height (cm)	91.92±4.07	72.16±5.05**	101.9±4.27	91.0±3.46**
Panicle length (cm)	22.56±1.21	12.75±0.61**	21.00±1.58	17.23±0.62**
No. of primary branch per panicle	10.90±0.67	10.95±0.81	10.81±0.92	11.32±0.67
No. of secondary branch per panicle	36.85±6.07	29.35±9.01**	41.34±4.55	43.33±5.83
Grain no. per panicle	187.4±16.63	166.4±14.17**	186.0±21.66	209.8±27.32*
Grain length (mm)	9.47±0.33	6.92±0.31**	10.14±0.35	8.47±0.43**
Grain width (mm)	2.63±0.13	3.24±0.11**	2.72±0.10	3.27±0.11**
Grain thickness (mm)	0.20±0.05	0.21±0.08**	2.19±0.05	2.30±0.07**
1000-grain weight (g)	24.63±0.08	20.72±0.18**	29.63+0.15	28.72+0.18**

*, ** Significantly different from the wild-type at 5% and 1% levels, respectively.