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ORIGINAL PAPER

Identification and characterization of the erect-pose panicle gene *EP* conferring high grain yield in rice (*Oryza sativa* L.)

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Abstract The breeding of *japonica* varieties with erect-pose panicle (EP) has recently progressed in the northern part of China, because these varieties exhibit a far higher grain yield than the varieties with normal-pose panicle (NP). A genetic analysis using the F_2 population from the cross between Liaojing5, the first *japonica* EP variety in China, and the Japanese *japonica* NP variety Toyonishiki revealed that EP is governed by a single dominant gene *EP*. Based on previous studies, map-based cloning of *EP* locus was conducted using Liaojing5, Toyonishiki, their F_2 population, and a pair of near-isogenic lines for *EP* locus (ZF14 and WF14) derived from the cross between the two varieties; consequently, the STS marker H90 was found to completely cosegregate with panicle pose. The H90 is located in the coding sequence AK101247 in the database, and the AK101247 of Liaojing5 has a 12 bp sequence in exon 5 replaced with a 637 bp sequence of its wild type allele. It was therefore considered that the AK101247 encodes the protein of the wild type allele at *EP* locus, and that the sequence substitution in exon 5 of Liaojing5 is crucial for expression of the EP phenotype. The effects of *EP* gene on agronomic traits were investigated using two pairs of near-isogenic lines (ZF6 vs. WF6 and ZF14 vs. WF14) derived from the cross between the two varieties. Experimental results showed that *EP* gene markedly enhanced grain yield, chiefly by increasing number of secondary branches and number of grains on the secondary branch. *EP* gene also produced a remarkable increase in grain density.

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Introduction

Improvement of grain yield is a perpetual goal in rice (*Oryza sativa* L.) breeding. It is well known that the development of stiff- and short-culmed (semidwarf) rice varieties and F_1 hybrid varieties has dramatically increased grain yield. Recently, another dramatic advancement in grain yield has occurred in the northern part of China, in a breeding program aiming to develop high-yielding varieties with erect-pose panicle (EP hereafter).

Panicle shape, which is associated with grain density, panicle length, grain number, and other features, is one of the target characteristics in rice breeding because of its strong association with grain yield. EP, however, has previously been considered an unfavorable trait in commercial varieties: a large majority of the former elite varieties have curved-pose panicle (normal-pose panicle, NP hereafter). The first *japonica EP* variety Liaojing5 in China was developed at the Institute of Agricultural Sciences in Liaoning Province (released in 1976), using the Italian *japonica* variety Balilla as the genetic source of the EP phenotype. Recently, Liaojing5 and its derivatives have been used as genetic sources in the development of high-yielding EP varieties in China.

Erect-pose panicle seems to be favorable in that it enhances the photosynthetic efficiency of the lower leaves, improves canopy temperature and humidity conditions, and increases CO_2 circulation around the plant. For these

reasons, EP is now regarded as one of the more significant characteristics in *japonica* rice breeding in China (Chen et al. 2001; Xu et al. 2005). This indicates the necessity of understanding the genetic and molecular basis of EP.

To date, two mapping studies have aimed at the isolation of the gene(s) involved in EP (Kong et al. 2007; Yan et al. 2007). Both found that the most significant gene is located on the long arm of chromosome 9. Kong et al. (2007), using the F_2 population from the cross between Liaojing5 and NP variety Toyonishiki, indicated that the gene was flanked by the SSR (simple sequence repeat) markers RM5686-23 and RM5833-11, and named the gene *EP*. Yan et al. (2007) detected two QTLs responsible for panicle curve-angle, using the doubled-haploid population derived from the cross between the Chinese EP variety Wuyunjing8 and the Chinese NP variety Nongken 57. Of the two, the *qPE9-1* with a higher LOD score was located between the STS (sequence tagged site) marker H90 and the SSR marker RM5652. On the basis of their chromosome positions and their effects on panicle pose, *EP* and *qPE9-1* seem to be the same locus.

In the present study, we first investigated the inheritance of EP in Liaojing5 and identified the candidate sequence of the gene conferring EP by narrowing down the region of *EP* locus based on the information provided in two previous works on EP (Kong et al. 2007; Yan et al. 2007). We also evaluated the effects of *EP* gene on grain yield and its related characteristics, such as number of panicles/m^2 and number of grains per panicle.

Materials and methods

Genetic analysis of the EP trait

The F_2 population, comprising 4,087 plants, derived from the cross of Liaojing5/Toyonisihi was subjected to a genetic analysis of the EP trait. Liaojing5 was the first EP variety in China, which was derived from the cross between the Italian variety Balilla with EP and the Japanese variety Toyonishiki with NP. F_2 seedlings were transplanted into the paddy field of Kyoto University in Kyoto, Japan, 30 days after sowing. Parental lines were planted together with their F_2 plants. Sowing was conducted on 13 May 2006. Fertilizers applied were 60, 90, and 90 kg/ha for N, P_2O_5, and K_2O, respectively. Plant spacing was 10×30 cm. Panicle pose was recorded for each F_2 plant. The goodness of fit of the observed segregation ratio to the expected was examined by the chi-square test.

Map-based cloning of *EP* gene

Genomic DNA was extracted from the leaves of each F_2 plant using TPS buffer (Monna et al. 2002). In PCR analysis, primers were designed with Primer3 in the GENETYX ver. 7.0 (GENETYX Co., Tokyo, Japan) based on the information obtained from the Genbank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). The PCR mixture (10 μl) contained 20 ng of genomic DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton ×-100, 5% (v/v) DMSO, 500 nM of each primer pair, and 0.2U Ex-Taq DNA polymerase (Takara Bio. Co., Shiga, Japan). PCR conditions were as follows: initial denaturation at 95°C for 3 min followed by 30 cycles of polymerization reaction, each consisting of a denaturation step at 98°C for 10 s, an annealing step at 55–60°C for 30 s, and an extension step at 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were subjected to electrophoresis on either 1% (or 2%) agarose gel or 6% polyacrylamide gel, and stained with ethidium bromide. Signals were detected with a UV transilluminator for agarose gel and a Molecular Imager FX system (BioRad Lab., CA, USA) for polyacrylamide gel. For sequencing analysis, amplified DNA fragments were purified from agarose gel using the EZ-10 Spin Column DNA Gel Extraction Kit (Bio Basic Inc., ON, Canada). The purified DNA fragments were sequenced with the GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, CA, USA) using a CEQ8000 Genetic Analysis System (Beckman Coulter, CA, USA).

Confirmation of the genotype of the H90 marker in other varieties

The above experiment indicated that *EP* gene includes the region of the STS marker H90 developed by Yan et al. (2007). To confirm this, we investigated the genotype of the H90 marker in 12 EP varieties (Shennong265, Shennong9741, HA5, Liaojing287, Liaojing326, Shen191, Kuayue, Balilla, Jida2004, Huadan995, Xing10, and Yifeng7) and 12 NP varieties (IR36, 9311, Kasalath, Hejiang18, Tiejing4, Liaojing371, Liaoyan16, Sifeng43, Akihikari, Lijiangxintuanheigu, 02428, and Nipponbare). Genomic DNA was extracted from the leaves of the four-leaf stage seedlings. The methods for detecting polymorphism of the H90 marker were similar to those used in map-based cloning of *EP* gene.

The proteins encoded by *EP* gene and its wild type allele were predicted based on the Nipponbare sequences obtained from Genbank.

Analysis of the effects of *EP* gene on agronomic traits

The effects of *EP* gene on unhulled grain yield (grain yield hereafter) and its related traits, viz. number of grains per panicle, number of filled grains, filled grain percentage, 1,000-grain weight (g), number of primary branches per panicle, number of secondary branches per panicle, number of grains on the primary branch, number of grains on the secondary branch, grain density (grains/cm), and number of

panicles/m^2, were investigated by comparing *EP*-homozygous lines (*EP/EP*) with their respective wild type counterparts (+/+), namely, ZF6 (*EP/EP*) with WF6 (+/+) and ZF14 (*EP/EP*) with WF14 (+/+). We also investigated the heterozygous advantages of *EP* gene on the same traits using the F_1 population (*EP*/+) from the cross of WF14/ZF14. ZF6 and WF6 were the near-isogenic pair derived from a single *EP*-heterozygous F_4 plant of the cross of Liaojing5/Toyonishiki, and ZF14 and WF14 were the near-isogenic pair derived from a single *EP*-heterozygous F_{12} plant of the same cross. Seedlings of these four lines were planted in the paddy field of Shenyang Agricultural University in Shenyang, China, 30 days after sowing. Field experiment was conducted with a randomized block design with three replications (one plot: 2 m × 4 m). Sowing was conducted on 10 April 2007. Fertilizers applied were 60, 90, and 90 kg/ha for N, P_2O_5, and K_2O, respectively. Plant spacing was 13 cm × 30 cm. Comparison of means was performed with Turkey's test using the SPSS ver.11.5 for Windows.

Results

Genetic analysis of the EP trait

A conventional genetic analysis of panicle pose was conducted using the F_2 population of the Liaojing5/Toyonishiki cross. The F_2 population, comprising 4,087 plants, was clearly divided into EP and NP groups. The ratio of EP type (3,076 plants): NP type (1,011 plants) fit the 3:1 ratio expected for one-locus segregation ($\chi^2 = 0.15$, $0.75 > P > 0.50$). Thus it was confirmed that EP is governed by a single dominant gene *EP*, as Kong et al. (2007) reported.

Map-based cloning of *EP* gene

Kong et al. (2007) indicated that *EP* locus was located between two SSR markers, RM5833-11 and RM5686-23 (348 kb), on the long arm of chromosome 9, with genetic distances of 1.5 and 0.9 cM, respectively (Fig. 1). Shortly afterward, Yan et al. (2007) detected a QTL, *qPE9-1*, responsible for the variation in panicle curve-angle was located between the STS marker H90 and the SSR marker RM5652 (21.8 cM) on the long arm of chromosome 9 (Fig. 1). We first searched for nucleotide-sequence polymorphism in the region between RM5833-11 and RM5686-23 (384 kb) among Liaojing5, Toyonishiki, ZF14 and WF14. Twenty-five SSR markers have been developed in this region (http://www.gramene.org/), but none of them proved to be polymorphic between Liaojing5 and Toyonishiki. Accordingly, we proceeded to sequence all the putative genes located in this region and determined about 80% of the total length of those genes. Consequently, we found one SNP (single nucleotide polymorphism, Liaojing5: T, Toyonishiki: C) at the 16,097,831 position in AP008215 (the accession of the whole sequence of chromosome 9), and one INDEL (insertion and deletion) polymorphism (10 bp deletion in Toyonishiki) at the 16,097,908–16,970,917 position in AP008215. However, they were not polymorphic between ZF14 and WF14, and both regions originated in Toyonishiki. These results indicated that *EP* locus is not located in the 384 kb region.

In the F_2 population from the Liaojing5/Toyonisihiki cross, comprising 423 plants, the genotype of H90 and the phenotype of panicle pose completely cosegregated, and no recombinants appeared. Around the region of H90, we observed one SNP (Liaojing5: G, Toyonisiki: A) at the 3′ terminal position of AK111616 (the 16,335,736 position in AP008215) between parents. However, the genotype of AK111616 was the Toyonisiki type in both ZF14 and WF14. On the other hand, both ZF14 and WF14 proved to have a Liaojing5 type allele at the SSR marker RM24428, which is closely linked to H90. We therefore concluded that *EP* locus was located within the 51 kb-region, including H90, between AK111616 (Os09g0441400) and RM24428 (Fig. 2a).

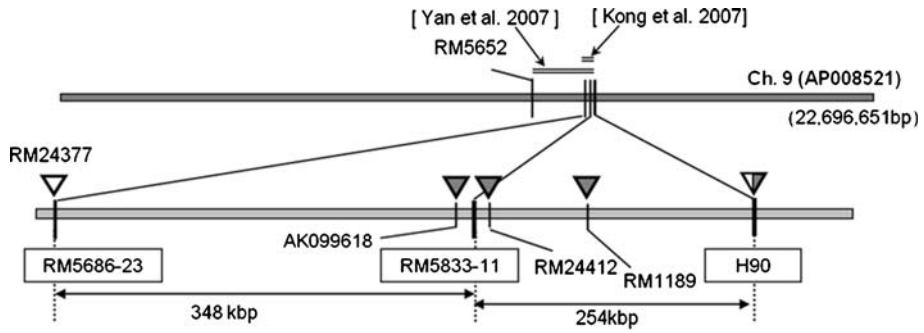

Fig. 1 Candidate chromosome regions (*doubled bars*) of *EP* gene indicated by previous reports (Kong et al. 2007; Yan et al. 2007) and the genotypes of a near-isogenic pair, ZF14 and WF14, at the SSR markers in these regions. White and black triangles indicate ZF14- and WF14-type marker genotypes, respectively. The *black* and *white triangle* indicates that the marker genotype is heterozygous

A database search (http://rapdb.dna.affrc.go.jp/) detected four coding sequences, Os09g0441400, AK241119, AK101247, and AK111977 within the 51 kb-region (Fig. 2b). Among them, AK111977 did not show polymorphism between Liaojing5 and Toyonishiki. Os09g0441400 and AK241119 showed polymorphism between Liaojing5 and Toyonishiki, and the genotypes of these markers of ZF14 were identical with those of Liaojing5, and those of WF14 were identical with those of Toyonishiki (Table 1). However, the polymorphic regions were found in introns of those two genes. The polymorphism in exon region between parents was found only in AK101247. Furthermore, we sequenced all four estimated coding regions and their flanking regions of ZF14 and compared those sequences with those of Nipponbare. But we could find no polymorphism between ZF14 and the NP variety Nipponbare except AK101247. These results strongly suggested that *EP* locus is AK101247.

Confirmation of *EP* locus polymorphism among other varieties

The sequence data on AK101247 for Liaojing5 and Toyonishiki showed that the two primers for H90 are located on the two sides of the polymorphic site in AK101247, respectively (Fig. 2c). The PCR products of Liaojing5 and Toyonishiki were 543 and 1,168 bp lengths, respectively, which were not consistent with the data reported by Yan et al. (2007). They stated that EP and NP varieties produced 1,168 and 543 bp length fragments, respectively.

All the EP varieties produced the same PCR fragments that Liaojing5 produced, whereas all the NP varieties

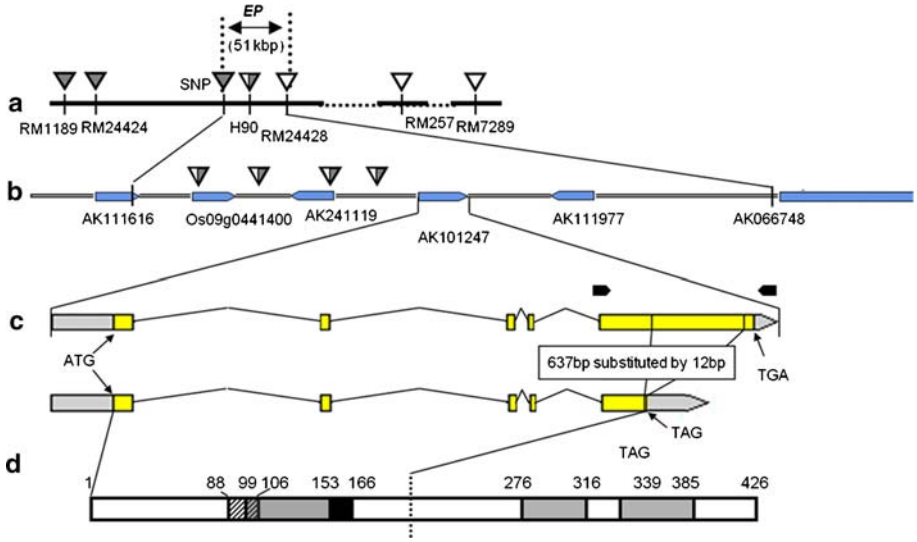

Fig. 2 Schematic representation of the molecular map and cDNA construct for the region of *EP* on rice chromosome 9. **a** Genotypes of ZF14 and WF14 for the SSR markers. *White* and *black triangles* indicate ZF14- and WF14-type marker genotypes, respectively. *Black* and *white triangles* indicate that the marker genotype is heterozygous. **b** The coding sequences (http://rapdb.dna.affrc.go.jp/) in the flanking region of the *EP* gene. **c** Exon–intron structure of AK101247 (*upper*) and the Liaojing5 allele (=the ZF14 allele) (*lower*). *Shadowed boxes* represent exons, and *lines* separating the *boxes* represent introns. **d** Schematic diagram of *EP* protein structure. The *black*, *striped*, and *gray boxes* indicate the 4-disulphide core domain of whey acidic proteins, the transmembrane region, and the VWFC domains, respectively. *Numbers* represent the positions of amino acids in the primary structure

Table 1 Nucleotide polymorphisms of three coding sequences considered as candidate cDNA clones of the *EP* gene

Coding sequence	Polymorphism[a]		
	Position [in AP008215]	Liaojing5	Toyonishiki
Os09g0441400	5′ UTR [16,340,362]	(A)	T substitution
	Downstream of 3′ UTR [next to 16,347,699]	(−)	AG insertion
AK241119	Intron [16,350,253]	(G)	A substitution
	Intron [16,352,237]	(A)	C substitution
AK101247	Fifth Exon [16,360,357–16,360,993]	637 bp substituted by 12 bp 'AGATCCTTTTTT'	(637 bp sequence) deletion and addition

[a] The nucleotides in parentheses are identical to the sequence AP008215 of the variety Nipponbare

produced the same fragments that Toyonishiki produced (Fig. 3). The EP original variety Balilla also produced the same fragment that Liaojing5 produced. These results suggest that AK101247 is the coding sequence of the wild type (NP-type) allele at *EP* locus.

The ORF sequence of the Toyonishiki allele (wild type allele) at the *EP* locus is considered to be identical with that of the Nipponbare allele, which harbors five exons with a 1,833 bp transcript length encoding 426 amino acids (aa), because these two varieties are closely related to one another. Compared with the Nipponbare nucleotide sequence, the *EP* gene in Liaojing5 harbored an early stop codon produced by the sequence substitution in exon 5, which leads to a 234aa truncation in the C-terminus of the predicted protein (Fig. 2d). The occurrence of a premature stop codon confers a high probability that a partial or complete loss of function will be induced; therefore, the truncated protein produced by the *EP* gene was considered to confer EP.

A predicted protein-sequence analysis showed that AK101247 harbored several known regions and domains (http://www.ebi.ac.uk/InterProScan/): a 4-disulphide core domain of whey acidic proteins at aa 156–166 (Simpson and Nicholas 2002), a transmembrane region at aa 88–106, and three von Willebrand factor type C (VWFC) domains (Voorberg et al. 1991; Colombatti et al. 1993) at aa 99–153, 276–316, and 339–385 (Fig. 2d). On the other hand, the *EP* gene of Liaojing5 harbored a 234aa truncation, resulting in the loss of two VWFC domains in the C-terminus. These results indicate that the EP phenotype is expressed due to a partial or complete loss of function of these protein motifs (Fig. 2d).

Analysis of the effects of *EP* gene on agronomic traits

Table 2 shows the effects of *EP* gene on agronomic traits. The EP lines, ZF14 and ZF6, both exhibited higher grain yields than their respective NP counterparts, WF14 and WF6. The F_1 population of the cross of ZF14/WF14 showed a higher grain yield than its EP parent ZF14. The effect of *EP* gene on number of panicles/m^2 was not significant between ZF14 and WF14. But ZF6 showed significantly fewer number of panicles/m^2 than WF6. The F_1 population of the cross of ZF14/WF14 showed a larger number of panicles/m^2 than both parents. This may suggest that *EP* gene exhibit a heterozygous advantage (over dominance) for number of panicles/m^2.

The EP lines showed larger numbers of grains per panicle and larger numbers of filled grains per panicle than their

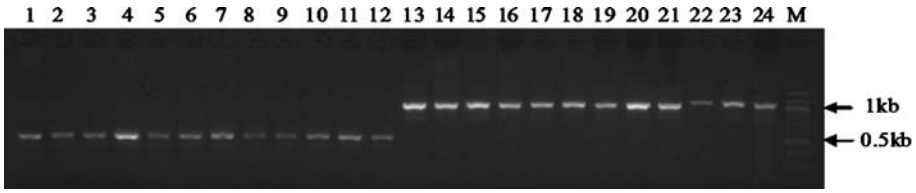

Fig. 3 Electrophoresis signals of the STS marker H90 in 12 erect-pose panicle (EP) varieties and 12 normal-pose panicle (NP) varieties. *Lanes 1–12* EP varieties, *lanes 13–24* NP varieties. *1* Shennong 265, *2* Shennong 9741, *3* HA5, *4* Liaojing287, *5* Liaojing 326, *6* Shen 191, *7* Kuayue 5, *8* Balilla, *9* Jida2004, *10* Huadan 995, *11* Xing 10, *12* Yifeng 7, *13* IR36, *14* 9311, *15* Kasalath, *16* Hejiang 18, *17* Tiejing 4, *18* Liaojing 371, *19* Liaoyan 16, *20* Sifeng 43, *21* Akihikari, *22* Lijiangxintuanheigu, *23* 02428, *24* Nipponbare, *M* 100 bp DNA ladder marker

Table 2 Comparison of grain yield and panicle-related traits between erect-pose panicle lines and normal-pose panicle lines

Traits	ZF14	WF14	ZF6	WF6	F_1-(WF14/ZF14)
Grain yield (g/m^2)	559.7c	508.1d	605.2b	558.9c	664.2a
Number of grains per panicle	137.2b	113.2d, e	145.6a	107.7e	125.1c
Number of filled grains per panicle	126.0a	106.3c	131.8a	101.0c, d	113.8b, c
Filled grain percentage	91.9a, b	93.9a	90.5b	93.8a	91.1b
1,000 grain weight (g)	22.9b	25.3a	22.5b, c	25.6a	23.0b
Number of primary branches per panicle	11.5b	11.3b	12.5a	11.0b	11.0b
Number of secondary branches per panicle	23.9a	17.2c	24.9a	15.5c	21.1b
Number of grains on primary branch	66.4b	64.1b	72.8a	63.4b	62.9b
Number of grains on secondary branch	70.8a	49.2c	72.9a	44.4c	62.2b
Grain density (grains/cm)	8.4b	5.4d	9.4a	5.6d	7.4c
Number of panicles/m^2	291.4d	283.0d, e	304.9c	325.8b	382.2a

Comparison of means was carried out using Tukey's test. All statistical analyses were performed using the software Statistical Package for Social Sciences (SPSS 11.5 for Windows). Different letters in every line indicate significant difference at the 5% level

respective NP counterparts. For these two traits, the F_1 population exhibited intermediate values between parents. ZF14 showed a similar filled grain percentage to that of WF14, while ZF6 showed a lower filled grain percentage than WF6. Both EP lines showed smaller 1,000 grain weights than their respective NP counterparts. In addition, for these two traits, the F_1 population exhibited similar values to those of ZF6. ZF6 exhibited a larger number of primary branches per panicle and a larger number of grains on the primary branch than WF6. However, neither of these effects was significant between ZF14 and WF14. In both comparisons, EP lines exhibited far more numbers of secondary branches per panicle and far more numbers of grains on the secondary branch. Number of primary branches per panicle and number of grains on the primary branch of the F_1 population did not differ from those of WF14, respectively. For number of secondary branches per panicle and number of grains on the secondary branch, the F_1 population exhibited intermediate values between parents. This suggested that *EP* gene has additive effects on these two traits. The lines can be ranked according to grain density as follows: ZF6 > ZF14 > F_1–WF6/ZF6 ≥ WF6 ≥ WF14. This order suggested that *EP* gene markedly increases grain density through enhancing the development of secondary branch and its grain.

Discussion

Improving the morphological and/or physiological characteristics of rice is one of the key factors in increasing its grain yield; therefore, the identification of genes that are closely involved in conferring such characteristics is quite important for rice breeding. The 'Green Revolution' in rice cultivation, for example, was made possible by the discovery and utilization of a semidwarf genetic resource (the semidwarfing gene *sd1*). This resource contributed to the dramatic increase in rice grain yield from the 1960s to the 1970s, which was especially great in tropical Asia (Futsuhara and Kikuchi 1997). Recently, EP has been used in rice breeding programs in the northern part of China, resulting in marked increases in grain yield per unit. A few studies have already demonstrated that EP is governed by a single dominant gene and modified by polygenes (Xu et al. 1995; Wang et al. 1997). In contrast, Zhu and Gu (1979) reported that EP was a recessive trait controlled by a single gene. Our experimental results clearly confirm that EP is governed by a single dominant gene *EP*, which is located on the long arm of chromosome 9. Using map-based cloning, we identified the *EP* candidate gene, which has a 12 bp sequence (AGATCCTTTTTT) in a position in exon 5 where the wild type allele has a 637 bp sequence (cDNA: AK101247). This genetic information will be very helpful in a breeding program aiming at developing high-yielding rice varieties using EP.

The EP lines, ZF14 and ZF6, both showed higher grain yields, more grains per panicle, more filled grains per panicle, more secondary branches per panicle, more grains on the secondary branch, and higher grain densities. However, 1,000-grain weight was lower in EP lines than in NP lines. As for number of primary branches per panicle and number of grains on the primary branch, a significant increase was observed in ZF6 over WF6. These increases were not observed in ZF14 over WF14, which indicates that these two traits are influenced by genetic background. Accordingly, although *EP*s ability to increase grain yield is chiefly attributable to its ability to increase number of secondary branches and number of grains on the secondary branch, there may be other genetic factor(s) responsible for the high grain yield seen in EP varieties.

The effects of the *EP* gene on grain density and secondary branch traits were quite similar to those of *Dn1* gene (*Dense panicle-1*), also located on the long arm of chromosome 9 (Nagao and Takahashi 1963). On the basis of their chromosome locations and their effects on panicle shape, *EP* and *Dn1* might be the same locus. Further analyses should be done to clarify the relationship between these two loci.

It is worth noting that the *EP*/+ plant showed a higher grain yield than the *EP/EP* parent. This suggests that the *EP*/+ heterozygote must be a useful genotype for high-yielding F_1 varieties. Other authors (Murai and Iizawa 1994; Murai et al. 2002) have observed this pattern in *Ur1* gene (*Undulate rachis-1*), which markedly increases grain yield by increasing the number of grains per panicle and the number of grains on secondary branches. Murai et al. (2003) reported that *Ur1*/+ plants exhibited higher grain yields than *Ur1/Ur1* plants. Because *EP* and *Ur1* are inherited independently of each other, it will be worthwhile to investigate the combination effect of these two genes on grain yield.

The sequence data of the NP variety Nipponbare predicted that the wild type allele corresponding to *EP* had a 426aa sequence that contained a 4-disulphide core domain, a transmembrane region, and three VWFC domains. Comparative sequence analysis indicated that the *EP* gene in Liaojing5 harbors a nonsense mutation in exon 5, which was induced by a nucleotide substitution. This mutation causes a 234aa truncation in the C-terminus of the predicted protein, which eliminated the last two VWFC domains. It is known that the putative VWFC domain is included in the protein of the fruit shape gene *OVETA* in tomato (Liu et al. 2002) and in that of the grain length gene *GS3* in rice (Fan et al. 2006). The *OVETA* gene harbors a putative bipartite nuclear localization signal, two VWFC domains, and a 1–70aa C-terminal domain, not only in tomato but also in

Arabidopsis and rice, and confers a pear-like shape. Since the *OVETA* gene harbors a premature stop codon, it functions as a recessive gene (Liu et al. 2002). The *GS3* gene, on the other hand, governs grain length, and harbors a putative PEBP-like domain, a transmembrane region, a putative TNFR/NGFR family cysteine-rich domain, and a VWFC domain. The long grain allele also harbors a premature stop codon and functions as a recessive gene (Fan et al. 2006). The similarity in amino acid truncations and resulting morphological changes among the rice erect panicle mutation, the tomato pear-shape mutation, and the long-grain rice mutation strongly suggests that, in each of them, the putative VWFC domain may play a role in regulating organ development. It is unclear why the *EP* allele functions as a dominant allele in spite of the elimination of two VWFC domains; further analysis is necessary to clarify this and other aspects of *EP* gene function.

In this study, we identified the *EP* candidate gene and elucidated its effects on several agronomic traits. The experimental results presented here will be very helpful in the production of high-yielding varieties with the EP phenotype.

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