

# Molecular tagging and mapping of the erect panicle gene in rice

Fan Na Kong · Jia Yu Wang · Ji Cheng Zou ·  
Li Xue Shi · De Min Jin · Zheng Jin Xu ·  
Bin Wang

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**Abstract** Erect panicle (EP) is one of the more important traits of the proposed ideotype of high-yielding rice. Several rice cultivars with the EP phenotype, which has been reported to be controlled by a dominant gene, have been successfully developed and released for commercial production in North China. To analyze the inheritance of the EP trait, we generated segregating F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> populations by crossing an EP-type variety, *Liaojing 5*, and a curved-panicle-type variety, *Fengjin*. Our results confirmed that a dominant gene controls the EP trait. Simple-sequence repeat (SSR) and bulked segregant analyses of the F<sub>2</sub> population revealed that the *EP* gene is located on chromosome 9, between two newly developed SSR markers, RM5833-11 and RM5686-23, at a genetic distance of 1.5 and

0.9 cM, respectively. Markers closer to the *EP* gene were developed by amplified fragment length polymorphism (AFLP) analysis with 128 AFLP primer combinations. Three AFLP markers were found to be linked to the *EP* gene, and the nearest marker, E-TA/M-CTC<sub>200</sub>, was mapped to the same location as SSR marker RM5686-23, 1.5 cM from the *EP* gene. A local map around the *EP* gene comprising nine SSR and one AFLP marker was constructed. These markers will be useful for marker-assisted selection (MAS) for the EP trait in rice breeding programs.

**Keywords** AFLP · *EP* gene · Molecular markers · SSR

## Introduction

The first breakthrough in increasing rice yield was the development of the semidwarf cultivar. The breeding of super-high-yielding rice lines has been the focus of rice breeders since the end of last century. One of the more important aspects for the breeding of super-rice lines has been the improvement of the plant type, denoted as ideotype breeding. Breeding programs aimed at developing dwarf or ideotype lines are mostly concerned with improving parts of the plant, such as the stem, leaf and tiller. More recently,

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F. N. Kong · L. X. Shi · D. M. Jin · B. Wang (✉)  
The State Key Laboratory of Plant Genomics,  
Institute of Genetics and Developmental Biology,  
Chinese Academy of Sciences, Beijing 100101, China  
e-mail: bwang@genetics.ac.cn

F. N. Kong  
The Graduate School of Chinese Academy of  
Sciences, Beijing 100039, China

J. Y. Wang · J. C. Zou · Z. J. Xu (✉)  
College of Agronomy, Shenyang Agricultural  
University, Shenyang 110161, China  
e-mail: xuzhengjin2005@yahoo.com.cn

however, panicle traits, such as spike number, panicle length and spike density, have received much attention, but to date little information on the erect panicle (EP) type has been reported.

In the 1960s, the EP phenotype in a wheat line was found to reduce the amount of shading and confer a high photosynthetic capacity, while curved panicles (CP) appeared to reduce the efficiency of the line with respect to the utilization of light energy (Yin 1961; Donald 1968). Twenty years later, an EP-type rice variety *Liaojing 5* with a high yield was developed and grown commercially (Xiong and Wang 1992). Subsequent studies have provided some evidence that rice EP-type plants can utilize solar energy effectively, improve ecological conditions, such as canopy illumination, temperature, humidity and CO<sub>2</sub>, and increase population growth rate, which would ultimately increase yield (Gao et al. 1999), while CP-type rice plants have been reported to lodge more easily than their EP counterparts (Du and Fang 2004). This led to the model of EP and super-high yield being the aim in *Japonica* breeding; validation of this strategy has been the successful development of another high-yielding EP-type variety, *Shennong 265*, which is successfully cultivated throughout the northern part of China.

Although the EP trait has been extensively propagated, there has been little advancement in the study of the *EP* gene. In the northern part of China, the *EP* gene mostly originates from *Balilla*, an old Italian variety that is representative of the EP type (Zhang et al. 2002). Field statistical results obtained from crossing EP-type *Liaojing 5* with CP-type *Fengjin* and *Shennong 129* indicated that a dominant gene controlled the *EP* trait (Xu et al. 1995). However, information on linkage markers and the chromosome location of the *EP* gene is currently lacking. Consequently, the objectives of this study were to tag and map the *EP* gene using molecular markers and to develop a linkage map of the area encompassing the *EP* gene. This information will provide valuable tools for marker-assisted selection (MAS) in rice EP breeding and further molecular cloning of the *EP* gene.

## Material and methods

### Plant materials

EP parent *Liaojing 5* was crossed with CP parent *Fengjin*. The progeny of this cross were used to develop an F<sub>2</sub> population, and a BC<sub>1</sub>F<sub>1</sub> population with CP parent *Fengjin*. Rice plants were cultivated in the experimental field of Shenyang Agricultural University under natural growing conditions.

### Scanning electron microscopy

Samples were prepared as described previously (Mou et al. 2000; Zhong et al. 1997; Zhong and Ye 1999), with some modifications. Briefly, stem tissues of rice panicles were excised with a razor and immediately placed in 2.5% glutaraldehyde, dehydrated in ethanol and then dried in a Samdri point dryer (Hitachi, Tokyo, Japan). The samples were coated with gold using E-1010 ion sputter (Hitachi) and observed with a scanning electron microscope (S-3000N; Hitachi).

### Statistical and genetic analyses

The numbers of EP plants and CP plants in the F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> populations were counted and calculated.  $\chi^2$  tests were performed to determine whether the goodness-of-fit was a ratio of 3:1 in the F<sub>2</sub> populations and a ratio of 1:1 in BC<sub>1</sub>F<sub>1</sub> populations.

### DNA isolation

Leaves from parent, BC<sub>1</sub>F<sub>1</sub> and F<sub>2</sub> plants were harvested and DNA extracted using the standard sodium dodecyl sulfate (SDS) method (McCouch et al. 1988). The DNA concentration was adjusted to 100 ng  $\mu\text{l}^{-1}$  with TE buffer (pH 8.0) for subsequent analyses.

### Simple sequence repeats (SSR) analysis

Bulked segregant analysis (BSA; Michelmore et al. 1991) was performed in conjunction with the SSR analysis. The SSR analysis was carried out in a 1× volume of reaction buffer containing

0.1 mM of each dNTP, 1.0 U *Taq* polymerase, 0.2  $\mu$ M primer and 20 ng template DNA; the final volume was adjusted to 20  $\mu$ l with ultra-pure water and one drop of mineral oil was added. The amplification reaction consisted of one cycle at 94°C for 4 min, followed by 35 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 1 min, with a final extension step at 72°C for 5 min. The amplification products were then separated on 6% polyacrylamide-gel electrophoresis (PAGE) sequencing gels at 85 W for 1.5 h after an initial pre-electrophoresis for 30 min. The gel was then removed from the apparatus and visualized by silver staining (Xu et al. 2002).

#### Amplified fragment length polymorphism (AFLP) analysis

AFLP (Vos et al. 1995) was performed in conjunction with BSA analysis following the manufacturer's instructions for the Gibco-BRL AFLP Analysis System kit (Life Technologies, Gaithersburg, Md.), with minor modifications (Chen et al. 2004). A total of 128 primer combinations were screened. The amplification products were separated on a 6% PAGE sequencing gel at 100 W for 2 h after an initial pre-electrophoresis for 30 min. The gels were then silver-stained, followed by SSR analysis.

#### Mapping of the EP gene

Primary mapping was performed by SSR analysis combined with BSA analysis, in which the mapped

SSR markers were used as anchor loci to map the *EP* gene. Following the identification of potential linkage markers, co-segregation analysis was carried out with the  $F_2$  population to verify the linkage of the markers to the *EP* gene. This method enabled the *EP* gene to be mapped primarily to a specific chromosome. In order to develop more markers closely linked to the *EP* gene, we performed AFLP analysis together with BSA analysis in the  $F_2$  population. Using the SSR and AFLP data together with the phenotypic data, we developed a local linkage map with MAP-MAKER ver. 3.0 (Lander et al. 1987). Recombination fractions were converted into genetic map distance (centiMorgans, cM) using the Kosambi mapping function (Kosambi 1944).

## Results

#### Observation of the EP-trait phenotype

The EP and CP types differed significantly with respect to phenotype. The panicles of both types remained equally erect from heading to flowering (Fig. 1a); however, the panicles of the CP-type cultivar started to droop towards the end of the flowering period. The maximum amount of curving in the CP-type cultivars occurred about 25 days after the start of flowering, while EP-type cultivars remained erect at this time (Fig. 1b, c). The leaves of the EP-type variety were greener and wider than those of the CP type. In addition, the culm nodule of the EP type was thicker than that of CP-type cultivars, resembling that of the

**Fig. 1** The phenotype of the erect panicle (EP)-type parent *Liaojing 5* and the curved panicle (CP)-type parent *Fengjin* at different growth stages. **a** The phenotype of EP and CP plants at the flowering stage, **b** the phenotype of an EP plant at the mature stage, **c** the phenotype of a CP type plant at the mature stage



culm dwarf. The EP type also had more primary branches and more spikelets per primary branch than the CP type (data was not shown).

#### Anatomical characterization of the two phenotypes

The panicle stem was used as experimental material to compare the anatomical structure difference between two phenotypes. Cell-wall morphology was examined by scanning electron microscopy. The EP type had more vascular bundles (Fig. 2a, b) and thicker sclerenchyma cell walls at the mature stage (Fig. 2c, d) than its CP counterpart; however, no difference in cell width and length were found between the two types.

#### Genetic analysis of the EP trait

Field tests performed once a year for two consecutive years showed that all of the F<sub>1</sub> individuals were of the EP type. Table 1 shows the genetic analysis of the progeny of the cross *Liaojing 5* × *Fengjin*. A total of 6342 plants from the F<sub>2</sub> population were examined over the 2 years of the study. Of these, 4846 were EP and 1496

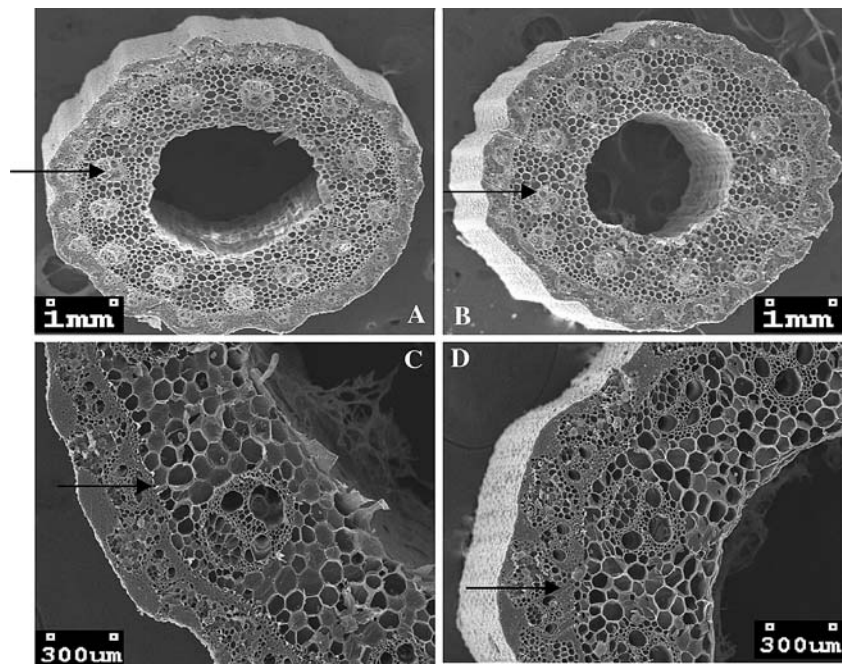
were CP, which fits a 3:1 ratio; this result was confirmed by  $\chi^2$  tests (Table 1), thereby indicating that a dominant gene controls the EP trait.

Similar observations on the BC<sub>1</sub>F<sub>1</sub> population generated from the same cross over the two consecutive years of the study confirmed the conclusion drawn from the F<sub>2</sub> data. Of a total of 227 BC<sub>1</sub>F<sub>1</sub> plants, 110 were EP and 117 were CP. This result ( $P > 0.05$ ; Table 1) fits the expected 1:1 ratio. It is therefore safe to conclude that the EP trait is controlled by one dominant gene. This conclusion is in agreement with that reported earlier by Xu et al. (1995) based on field statistical data.

#### Screening of SSR and AFLP markers linked to the EP gene and primary mapping of the EP gene

One hundred plants of the F<sub>2</sub> population derived from the cross *Liaojing 5* × *Fengjin* were used to screen for molecular markers putatively linked to the EP gene. In total, 120 SSR primer-pairs that were evenly distributed throughout the 12 chromosomes were selected and screened. The screened markers were further checked with 100 recessive individuals of the F<sub>2</sub> population to

**Fig. 2** Scanning electron micrographs showing the differences between vascular bundles and sclerenchyma cells in EP-type and CP-type plants. **a, b** Comparison of vascular bundles in EP (**a**) and CP (**b**)-type plants, **c, d** comparison of sclerenchyma cells in EP (**c**) and CP (**d**)-type plants





**Table 1** Genetic analysis and  $\chi^2$  scores of F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> populations generated from the cross *Liaojing 5* × *Fengjin*

Population	Year	Total number of plants	Number of plants with the erect panicle (EP) trait	Number of plants with the curved panicle (CP) trait	EP/CP ratio	P	$\chi^2$ -test
F <sub>2</sub>	2004	4834	3648	1186	3.08	>0.05	ns <sup>a</sup>
	2005	1258	948	310	3.06	>0.05	ns
	Total	6092	4596	1496	3.07	>0.05	ns
BC <sub>1</sub> F <sub>1</sub>	2004	139	65	74	0.88	>0.05	ns
	2005	88	45	43	1.05	>0.05	ns
	Total	227	110	117	0.94	>0.05	ns

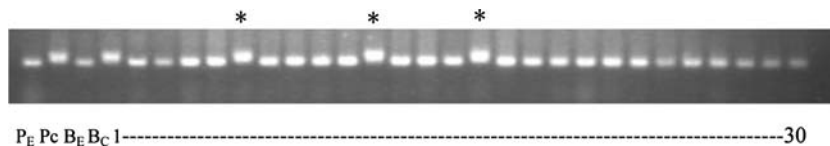
<sup>a</sup> ns, Means not significantly different

verify their linkage with the *EP* gene. Of these, one marker, RM242 on chromosome 9, showed linkage with the *EP* gene. Subsequently, all of the SSR markers flanking RM242 on the updated linkage map were analyzed, and five markers (RM1189, RM257, RM242, RM3787 and RM1013) were identified as being linked to the *EP* gene. The amplification pattern of RM257 is shown in Fig. 3.

In order to find new markers that are more closely linked to the *EP* gene, we synthesized SSR primers flanking the *EP* gene, based on the GRAMENE public database (<http://www.gramene.org>), and subsequently analyzed their linkage relationship to the *EP* gene. The results indicated

that four new SSR markers (RM5707-18, RM5093-5, RM5686-23 and RM5833-11; Table 2) were linked to the *EP* gene. The *EP* gene was located between the RM5686-23 and RM5833-11 markers, with genetic distances of 1.5 and 0.9 cM, respectively. According to the physical mapping GRAMENE data, the two markers were located on bacterial artificial chromosome (BAC) accessions AP005686 and AP005833, respectively, and the physical distance between RM5686-23 and RM5833-11 was about 400 kb.

In the AFLP-BSA analysis, about 50–80 bands appeared in each lane of the PAGE gel when amplified with a 2 + 3 primer-combination and visualized using silver staining (Xu et al. 2002). In



**Fig. 3** The amplification pattern of the SSR marker, RM257, in 30 F<sub>2</sub> plants of the CP type generated from the cross *Liaojing 5* × *Fengjin*. The samples in each lane are: P<sub>E</sub>, EP parent *Liaojing 5*, P<sub>C</sub>, CP parent *Fengjin*, B<sub>E</sub>,

EP bulk, B<sub>C</sub>, CP bulk, 1–30 F<sub>2</sub> CP individuals. A 3.5% agarose gel was used in the electrophoresis, and the gels were subsequently stained with EtBr. Asterisks indicate the recombinant

**Table 2** Newly developed SSR markers

Marker name	Originated BAC/PAC <sup>a</sup>	Primer sequence	Fragment size (bp)
RM5707-9	AP005707	5707-9F 5'GCATCGCTTGATTAGGCT3' 5707-9R 5'CCCACATCCAACAGCATT3'	240
RM5093-5	AP005093	5093-5F 5'GGACTACCAATCTTGAGTTT3' 5093-5R 5'ATGTCTGGTGTAATTGAGA3'	286
RM5686-23	AP005686	5686-23F 5'CCATTATTAGGTCGGACG3' 5686-23R 5'TTGGTTGTTTCGGTGGAAT3'	320
RM5833-11	AP005833	5833-11F 5'GAACCACACCAACCAACT3' 5833-11R 5'GATTTCTCTATGATGACTGGC3'	335

<sup>a</sup> BAC, Bacterial artificial chromosome; PAC, P1-derived artificial chromosome

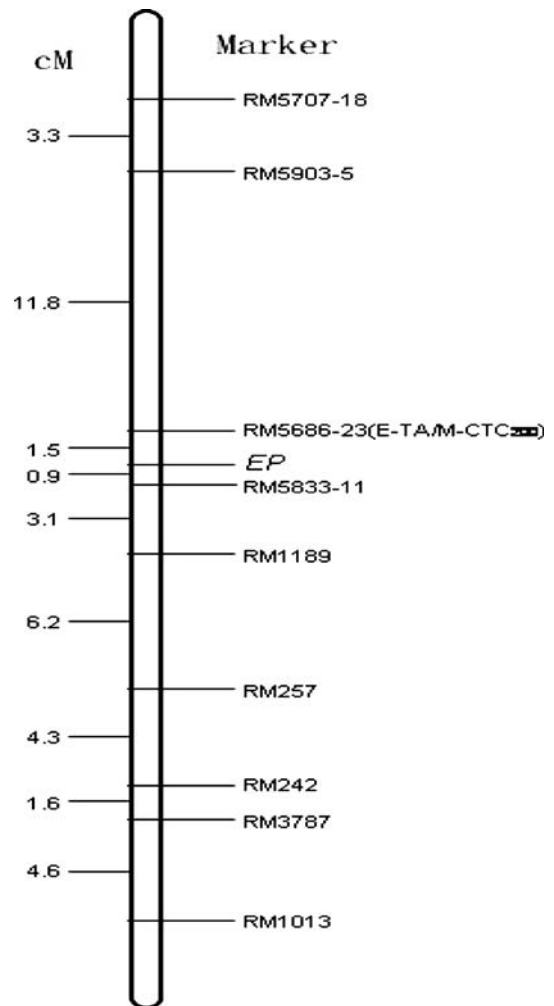
total, 128 primer-combinations were screened, of which three combinations (E-TA/M-CTC, E-AG/M-CGA, E-TA/M-CGG) generated three markers that were linked to the *EP* gene. The most closely linked marker, E-TA/M-CTC<sub>200</sub>, was mapped to the exact same location as the SSR marker, RM5686-23.

Construction of a local linkage map of the region encompassing the *EP* gene on chromosome 9

Based on the results of the linkage analysis, we determined that ten markers were linked with the *EP* gene, nine of which are SSR markers and only one is an AFLP marker. The phenotypic, SSR and AFLP data were combined for linkage analysis using MAPMAKER ver. 3.0. This analysis produced a local linkage map of the *EP* gene-encompassing region on chromosome 9 (Fig. 4). The most closely linked markers flanking the *EP* gene are RM5686-23 and RM5833-11, with genetic distances of 1.5 and 0.9 cM, respectively.

## Discussion

The EP-and CP-type rice plants differed distinctly from each other in both phenotype and anatomical structure. These differences were only seen after the flowering period was over – not during the heading period – which indicates that expression of the *EP* gene may occur during the stages of spikelet development. The two notable anatomical differences between the EP-type and CP-type plants in this study were that the former have more vascular bundles and thicker sclerenchyma cell walls than the latter. These two typical EP-type plant characteristics can provide mechanical strength by enhancing the mechanical strength of the stem towards the panicle, thereby allowing the panicle to stay erect more easily. These traits also may be beneficial to the whole population of rice plants by allowing the plants a better exposure to solar energy and enhancing photosynthesis in the reproductive growth period, thus increasing the ultimate rice yield (Jin et al. 2003). To date, the EP trait is only found in the *Japonica* super-high-yielding cultivar, with the CP



**Fig. 4** Molecular linkage map of the rice chromosome 9 showing the location of the *EP* gene. The linkage distance between the *EP* gene and the linkage markers RM5686-23 and RM5833-11 was 1.5 and 0.9 cM, respectively

type being the ideotype of the *Indica* high-yielding cultivar in South China. When the EP-type cultivar was grown in South China, the seed setting rate was reduced. It would therefore appear that the choice of EP or CP depends on the different growth conditions and growth areas.

The EP-type cultivar, with a high spikelet density, usually tends to suffer from panicle disease, such as false smut. However, Chen (2003) mentioned that the panicle curvature was negatively associated with spikelet density and incidence of false smut. The correlation coefficient with incidence of false smut was too low to

discern the difference level, which indicated that no direct relation existed between erect panicle trait and incidence of false smut. Subsequent studies confirmed that the incidence of false smut was correlated with high spike density, which is the primary causal factor of severe false smut in the EP-type cultivar. Therefore, in rice breeding of the EP-type cultivar, reducing the spikelet density properly could completely decrease the incidence of false smut.

The *EP* gene, which originated from *Balilla*, was always thought to be a single dominant gene or an additive major gene plus a minor gene (Wang et al. 1997; Xu et al. 1995). In this investigation, *Liaojing 5*, the donor of the *EP* gene, also originated from *Balilla*; it was crossed with *Fengjin*, and thought to have a single dominant gene. Genetic analysis of the F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> progeny confirmed this. The results from chromosome mapping of the EP trait obtained here will be useful for further molecular cloning of the *EP* gene through the map-based cloning approach. A large number of studies have shown that the panicle curvature was negatively related with the number of primary branch and primary branch grains and positively related with panicle length, yet the correlation coefficient did not reach the difference level. Whether the relation between EP trait and other traits is due to the pleiotropic effects of the *EP* gene or to linkage to the *EP* gene is still to be investigated.

This study is the first to report the chromosome mapping of the *EP* gene. Ten markers were linked to *EP* gene, which was mapped to the long arm of chromosome 9. These two closely linked markers, SSR markers RM5686-23 and RM5833-11, will be useful for MAS of the EP trait in breeding programs.

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