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ORIGINAL PAPER

Identification and characterization of a major QTL responsible for erect panicle trait in *japonica* rice (*Oryza sativa* L.)

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Abstract Panicle erectness (PE) is one of the most important traits for high-yielding japonica cultivars. Although several cultivars with PE trait have been developed and released for commercial production in China, there is little information on the inheritance of PE traits in rice. In the present study, 69 widely cultivated *japonica* cultivars and a double haploid (DH) population derived from a cross between a PE cultivar (Wuyunjing 8) and a drooping panicle cultivar (Nongken 57) were utilized to elucidate the mechanisms of PE formation and to map PE associated genes. Our data suggested that panicle length (PL) and plant height (PH) significantly affected panicle curvature (PC), with shorter PL and PH resulting in smaller PC and consequently more erect. A putative major gene was identified on chromosome 9 by molecular markers and bulk segregant analysis in DH population. In order to finely map the major gene, all simple sequence repeats (SSR) markers on chromosome 9 as well as 100 newly developed sequence-tagged site (STS) markers were used to construct a linkage group for quantitative trait locus (QTL) mapping. A major QTL, *qPE9-1*, between STS marker H90 and SSR marker RM5652, was detected, and accounted for 41.72% of PC variation with pleiotropic effect on PH and PL. another QTL, *qPE9-2*, was also found to be adjacent to *qPE9-1*. In addition, we found that H90, the nearest marker to *qPE9-1*, used for genotyping 38 cultivars with extremely erect and drooping panicles, segregated in agreement with PC, suggesting the H90 product was possibly part of the *qPE9-1* gene or closely related to it. These data demonstrated that H90 could be used for marker-aided selection for the PE trait in breeding and in the cloning of *qPE9-1*.

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Introduction

Semi-dwarf rice breeding began in the 1960s has significantly contributed to the improvement of rice yield potential. Semi-dwarf varieties have been associated with high yield due to their improved resistance to lodging, which resulted from a significant increase in total biomass under conditions of increased fertility. Additionally, the relatively shorter plant height (PH) facilitated the transfer of photosynthesis products to grain yield.

High-yield cultivars are not only associated with PH, but also require feasible tilling ability, thick and erect leaves and robust roots. In particular, leaf position plays an important role in canopy photosynthesis efficiency after heading (Donald 1968; Peng et al. 1994; Khush 1996; Yuan 1997; Chen et al. 2001). Conventional cultivars usually exhibit a drooping panicle after the grain filling stage. The panicle is the top organ in rice and is, consequently, an important

component of the canopy. Previous research suggested that although rice panicle had an important effect on photosynthesis efficiency of canopy during the grain filling stage, it has little effect on grain filling itself (reviewed by Xu et al. 1995a).

Guihuahuang, a panicle erectness (PE) cultivar, was developed in the early 1960s by Taihu Institute of Agricultural Sciences in Jiangsu province of China, by pedigree selection from a Balilla hybrid progeny. This cultivar exhibited higher yield potential compared to other common *japonica* varieties due to its erect panicle and leaves, which significantly optimized the canopy structure. The following decades witnessed the development and release of additional cultivars with erect or semi-erect panicles, including two well-known PE cultivars, Qianchonglang, developed by the Shenyang Agricultural University, and Liaojing 5, developed by the Institute of Agricultural Sciences in Liaoning Province. Most PE cultivars were derived from the cross of Balilla or Balilla-derived varieties with other parents. Since the 1980s, a greater number of high-yielding *japonica* varieties with PE have been released in China, leading to a dramatic increase in the acreage of PE varieties (Zhang et al. 2002a). Currently, planting area under *japonica* rice ranges from the Yangzi River to the Songliaon plain, mostly with high-yield PE varieties.

In spite of extensive development and cultivation of PE varieties, the mode in PE inheritance has not been fully elucidated. While Zhu and Gu (1979) reported that PE was a recessive trait controlled by a recessive gene, others (Xu et al. 1995b; Wang et al. 1997) reported that PE was controlled by a major gene, with dominant or additive effects, and modified by polygenes. Kong et al. (2007) further assigned the dominant gene *EP* on chromosome 9, between two newly developed simple sequence repeats (SSR) marker, RM5833-11 and RM5833-23, at the genetic distance of 1.5 and 0.9 cM, respectively. However, research is still needed to investigate the mode of inheritance, as well as the genetic effect of genes that mediate PE, and their positions on chromosomes, so as to improve this trait in rice breeding.

The understanding of the relationship between molecular marker and genes (quantitative trait locus, QTLs) is facilitated by a large number of molecular markers available, such as SSR markers, and the availability of the entire genome sequence data for *japonica* and *indica* species. In the current study, 69 japonica varieties, including erect, semi-erect and curved panicle types, were used to investigate the mechanism of PE formation. Additionally, a double haploid (DH) population derived from hybrids of PE variety Wuyunjing 8 and drooping panicle variety Nongken 57, was generated to tag genes associated with panicle traits.

Materials and methods

Plant materials and mapping population

A DH population generated through anther culture from F_1 hybrids of a cross between erect panicle variety Wuyunjing 8 and drooping panicle variety Nongken 57 was employed to dissect the mechanism of erect panicle formation. For which, 128 lines were obtained in 2004, and then enlarged to 154 lines in 2005. In addition, 69 widely cultivated erect, semi-erect and drooping panicle *japonica* varieties were also selected to for PE trait analysis. All DH lines and *japonica* varieties were grown in the field in Yangzhou, China, during the summer of 2004 and 2005, with germinated seeds sown in the seedling nursery on May 10–15 each year. Seedlings were transplanted to paddy fields on June 18–20, with single plant per hill spaced at 20 × 30 cm. Thirty plants for each DH line and variety were planted in each year with three rows. Standard practices were followed for water and fertilizer management.

Phenotype data collection

All panicle traits were measured during the mature stage and included thousand-grain weight (TGW), panicle length (PL), seed set (SS), number of filling grains (NFG), number of primary branches (NPB), number of second branches (NSB), diameter of panicle neck (DPN), number of grains on primary branches (NGPB), number of grains on second branches (NGSB), average length of primary and second branches (ALPB and ALSB) and PH. Heading dates per plant were recorded followed by measurement of panicle curvature (PC) 20 days later, as described by Xu et al. (1995b). The PC was presented by the angle included between the line connecting panicle pedestal with panicle tip and the elongation line of stem. For all above traits, five representative plants of each DH line and variety in the middle of each plot were sampled, and the main stem panicle of each plant was chosen for trait measurement and further analysis.

DNA extraction and SSR and STS marker assay

Genomic DNA for each plant was isolated from fresh-frozen leaves using CTAB method (Rogers and Bendch 1988), and then dissolved in TE solution for storage and subsequent analysis. The PCR reaction mixture (total volume 20 μl), contained 20 ng template DNA, 10 mM Tris–HCL, 50 mM KCl, 2.5 mM MgCl2, 5 mM dNTP, 50 pmol primers and 1.0 U *Taq* polymerase, with reaction cycles as follows: one cycle (94°C, 4 min), 35 cycles (94°C, 45 s; 55°C, 45 s; 72°C, 2 min) and a final extension step (72°C, 5 min). PCR products were subjected to electrophoresis on either

1% agarose (bands >50 bp) or 6% polyacrylamide gel (bands <10 bp) and stained with ethidium bromide and silver-stain, respectively, for visualization.

Bulk segregant analysis

Bulk segregant analysis (Michelmore et al. 1991) was performed, in conjunction with SSR analysis, to identify genes underlying PC traits. A total of two bulks, made by mixing equal amounts of DNA from ten PE plants and ten drooping panicle plants of the DH population, were subjected to polymorphism screening using SSR and sequence-tagged site (STS) markers.

Linkage map construction and major QTL identification

Polymorphisms between the two parents Wuyunjing 8 and Nongken 57 were detected with 600 SSR markers randomly distributed on the 12 rice chromosomes and the polymorphic makers were subsequently used to identify polymorphisms between the two DNA pools. All polymorphic markers found between the two DNA pools were inferred to be linked with QTLs associated with PC. Also, based on the information of the location of polymorphic markers on rice chromosomes, additional SSR and STS markers were developed, according to the genomic sequence diversity between the *japonica* accession Nipponbare and *indica* accession 93–11 (Table 1). In detail, according to the information of SSR location, the corresponding BAC clones of Nipponbare were searched, and then use "BLASTn" to find the homologues BAC of 93–11, followed by "Alignment". The deletion/insertion region would be used to develop new STS markers. The sequences of STS primer were designed by using Primer Premier 5.0 software before being synthesized. Linkage map construction was performed by means of Mapmaker (version 3.0) (Lander et al. 1987).

Composite interval mapping (CIM) was implemented in Windows QTL Cartographer version 2.5 (Wang et al. 2006). For CIM, a 2-cM window size was used for the genome scans. The threshold LOD scores to declare significance at = 0.05 was estimated empirically with 300 permutations.

Statistical analysis

Regression analysis was performed with the SPSS software (version 10) to elucidate the mechanism of erect panicle formation based on the 2005 data of DH population and 69 varieties. Briefly, the PC trait was treated as dependent variable, and other agronomic traits were independent variables.

Analysis of variance of PC was performed by using the SAS system based on the 2 years data of 128 DH lines. On the basis of ANOVA, the heritability of PC was calculated as SSgenotype/(SSgenotype + SSerror) based on the DH population.

Results

PC performance in the DH population

The results of ANOVA for PC trait showed that, in the DH population, the variation of PC trait was mainly caused by genotype (Table 2), and the interaction between genotype and environment was observed also. The heritability of PC was 0.8538, indicating PC trait inherited stably, and mainly controlled by genetic factor. The frequency distribution of PC trait of DH lines showed that the PC trait segregated continuously, from the erect to the drooping panicles (erect panicle type, PC < 40°; semi-erect panicle type, 40° < PC < 50°; drooping panicle type, PC > 50°, Xu et al. 1995a, b), with two peaks observed at the extremes and fewer semi-erect panicle types in the center (Fig. 1), suggesting the PC trait was controlled by a major QTL, and possibly modified by few minor genes.

Table 1 Newly developed polymorphic STS markers between the two parents Wuyunjing 8 and Nongken 57

Name	BAC	Forward primer	Reverse primer	Expected length(bp)
H50	AP005574	5′-CTAACAGACAAACAAGCGAC-3′	5′-AGAGGGTGAATGGAAAGACT-3′	156
H58	AP006849	5′-CTTCTCTCTCCCTATTG-3′	5′-CCTCTGTTTTACACTTG-3′	181
H59	AP006849	5′-CCGTGTTCATTTGCTCATC-3′	5′-TTCGCCTCCTCTGCCGCT-3′	145
H90	AP005419	5′-TTCCCTGCAGATCAAAATTG-3′	5′-TTCAATGGTTCAACCTCG-3′	1,168
c7	AP005129	5′-GATGAATCCGCCGAGTGTA-3′	5′-AACCAAGCCAACCAGAGGG-3′	397
c15	AP005904	5′-GTAGTAGCAAATCCCAAGGC-3′	5′-AAAACTCCCATTCATCAAGC-3′	430
c28	AP005904	5′-TGGTTCACGCCGCCTTTG-3′	5′-CTATTTATGCTCTCTCACG-3′	265
c25	AP005904	5′-TTGCGTTGTTCACGACTC-3′	5′-ATGTGGGCTTTCACTGGC-3′	414

Table 2 Two-way analysis of variance for panicle curvature (PC) trait based on the DH population

S.O.V	*df*	SS	MS	*F*	Heritability
Genotype	127	902733	7108.13	48.092**	0.8538
Year	1	440.849	440.849	2.9827	
Genotype × year	127	124523	980.498	6.6339**	
Error	1046	154600	147.802		
Total	1301	1182297			

**Significant at 0.01 level

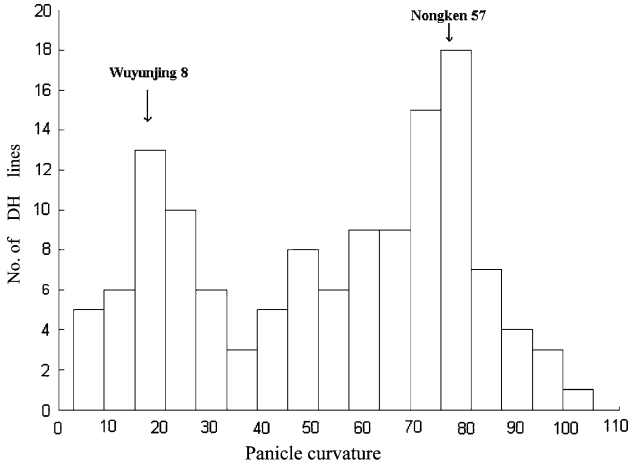

Fig. 1 The frequency distribution for average panicle curvature of DH lines in 2004 and 2005. (128 lines in total)

The relationship between PC and other panicle traits

Our data suggested that, in the DH population, PH, PL and NGPB significantly contributed to PC, with the largest effect from PL, suggesting that shorter PL and PH led to smaller PC, resulting in panicle erect. Additionally, PL and PH were also detected to contribute to PC in the 69 varieties, but PH has opposite effect to PC when compared to that in the DH population. The reason was possibly due to the variety sample selection. In 69 varieties, the PH of most PE varieties has been improved to fit the requirement of ideal plant type to achieve high yield level, whereas, most drooping panicle varieties were released some decades ago, and their PHs were less than 100 cm. Therefore, the PC was correlated negatively to PH in 69 variety sample. No significant effects on PC were detected from all other measured panicle traits, including TGW, SS, NFG, NPB, NSB, DPN, NGSB, ALPB and ALSB in both the DH population and the 69 cultivars (Tables 3, 4).

Polymorphic marker detection between two DNA pools

Surprisingly, of the 600 SSR markers initially screened, only 60 were polymorphic between the two parents and were selected for further analysis of polymorphism between the two DNA pools. SSR marker RM410, positioned on chromosome 9, was polymorphic between the two DNA bulks, suggesting RM410 could be putatively linked to a major PC QTL or gene (Fig. 2). In order to confirm this assertion, we divided the DH lines into two groups based on RM410 genotypes, and analyzed differences between both groups. Our data confirmed that a major QTL resided around the RM410 anchored region, as significant differences in PC were found between two groups (data not shown).

Table 3 Regression analysis for panicle curvature to other agronomic traits based on DH population

Trait	Unstandardized coefficients		Standardized coefficients	*t*-value	*P*-value
	B	Standard error	Beta		
TGW	−0.827	0.610	−0.085	−1.356	0.178
PH	0.528	0.190	0.247	2.780	0.006
PL	4.218	1.057	0.419	3.991	0.000
DPK	−9.500	12.611	−0.052	−0.753	0.453
NPB	−0.216	2.079	−0.011	−0.104	0.918
NSB	−0.478	0.510	−0.096	−0.937	0.351
NGPB	−1.077	0.346	−0.381	−3.112	0.002
NGSB	−0.317	0.263	−0.221	−1.207	0.230
ALPB	3.896	2.302	0.164	1.692	0.093
NFG	0.371	0.235	0.320	1.581	0.117
SS	33.863	19.772	0.106	1.713	0.090

Table 4 Regression analysis for panicle curvature to other agronomic traits based on 69 varieties

Trait	Unstandardized coefficients		Standardized coefficients	*t*-value	*P*-value
	B	Standard error	Beta		
TGW	−1.013	1.457	−0.075	−0.695	0.490
PH	−0.693	0.278	−0.256	−2.494	0.016
PL	7.082	1.752	0.635	4.041	0.000
DPN	−18.600	14.152	−0.154	−1.314	0.194
NPB	−0.798	2.160	−0.063	−0.369	0.713
NSB	−1.203	0.661	−0.492	−1.819	0.074
NGPB	−0.028	0.260	−0.017	−0.107	0.915
NGSB	0.281	0.203	0.344	1.387	0.171
ALPB	0.079	3.613	0.004	0.022	0.983
SS	4.697	26.475	0.020	0.177	0.860

Linkage map construction and QTL mapping

In order to precisely map the major QTL around RM410, and assess its genetic effect, additional polymorphic markers,

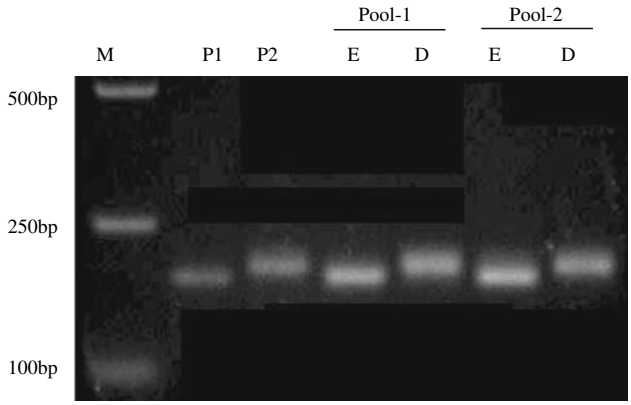

Fig. 2 The polymorphic pattern revealed by RM410 between the two parents and DNA bulk pools. P1, Wuyunjing 8; P2, Nongken 57; *E* erect-panicle; *D* drooping panicle. A 3.0% agarose gel was used in the electrophoresis, and the gel was subsequently stained with EtBr

including 12 SSR and 8 newly developed STS, were used to construct a linkage map, covering a length of 88.4 cM (Fig. 3). CIM showed a major QTL, named *qPE9-1*, located between the SSR marker RM5652 and STS marker H90 anchored interval, 1.8 cM from H90, accounting for 41.72% of the variations in PC. The allele from Nongken 57 increased PC by approximately 21.52°. Another QTL, *qPE9-2*, with the contribution of 25.97% of total variance, was also identified (Table 5).

Quantitative trait locus CIM was done to investigate PC, PH, NGPB and PL, given the significant relationship between these traits. Two QTLs, *qPH9-1* and *qPH9-2*, underlying PH trait and one QTL, *qPL9*, controlling PL trait, were identified. Interestingly, *qPH9-1* and *qPL9* associated with PH and PL, respectively, were in the same region where *qPE9-1* resided, with overlapping confidence intervals among them (Fig. 3 and Table 5). These results suggested that similar QTL on chromosome 9 might have contributed to PL, PH and PC simultaneously. No QTL was observed for NGPB at the *qPE9-1* locus.

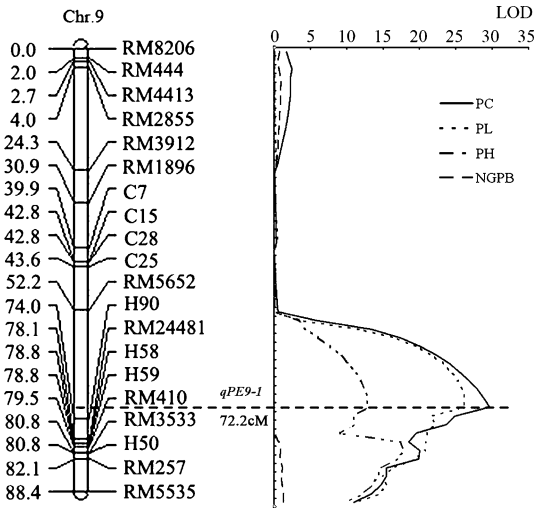

Fig. 3 Molecular linkage map of rice chromosome 9 showing the location of *qPE9-1*. The genetic distance (Kosambi, centiMorgan) and marker name are shown on the left and right of the chromosome, respectively. *PC* panicle curvature, *PL* panicle length, *PH* plant height, *NGPB* number of grains on the primary branches

The distribution of *qPE9-1* in widely cultivated japonica rice varieties

A survey of the distribution of *qPE9-1* in 38 cultivated japonica varieties, with extreme erect and drooping panicles, were performed by genotyping with QTL flanking markers RM24481 and H90 (Fig. 4). The results demonstrated H90 co-segregated with the panicle types, whereas random distribution was noted for both the erect and drooping panicles for RM24481, even though it was adjacent to H90. This may be due to the recombination between the RM24481 and H90 occurred during the development of PE varieties, and breeders had focused on the PE trait, no selection pressure on RM24481 locus. The result primarily suggested H90 was developed on the *qPE9-1* locus or closely linked to *qPE9-1*. Zhang et al. (2002a, b) reported that two-thirds of the varieties released with PE originated from an Italian variety Balilla. Our data suggested all PE varieties had the same H90 genotype, further confirming the PE trait was from Balilla.

Discussion

Panicle erectness, as one of important characteristic of high yield *japonica* varieties, has drawn increasing attention of rice breeders. More and more PE *japonica* varieties were developed and released for production. Currently, *japonica* PE variety is the predominant type cultivated in the Jiangsu Province, where the first PE variety "Guihuahuang" was released in 1964. Additionally, many PE varieties were released in Liaoning Province in northern, accounting for 55% of the varieties grown in the province (Zhang et al. 2002a, b). To date, *Japonica* varieties with PE phenotype have been widely cultivated in most of *japonica* growing regions, from Zhejiang to Liaoning Province in China.

In spite of the wide use and large numbers of high yield PE varieties currently cultivated, the inheritance of the PE phenotype has not been fully elucidated. Kong et al. (2007) firstly reported that, in Liaojing 5/Fengjin cross, a dominant gene *EP* was responsible for erect panicle traits, which was located on chromosome 9, between RM5833-11 and RM5833-23, with the genetic distance 1.5 and 0.9 cM, respectively. In the present study, a major QTL, *qPE9-1* on chromosome 9, was identified to be mainly responsible for

Table 5 The parameters of QTLs for panicle length, plant height and panicle curvature on chromosome 9

QTL	Chromosome	Marker interval	Peak position (cM)	LOD score	Additive effect	R^2
qPE9-1	9	RM5632-H90	72.21	29.66	−21.52	0.4172
qPE9-2	9	H58-H59	78.81	20.04	−17.74	0.2597
qPH9-1	9	RM5632-H90	72.21	12.93	−11.10	0.2922
qPH9-2	9	H58-H59	78.81	17.92	−9.18	0.4050
qPL9	9	RM5632-H90	72.21	26.09	−2.37	0.4650

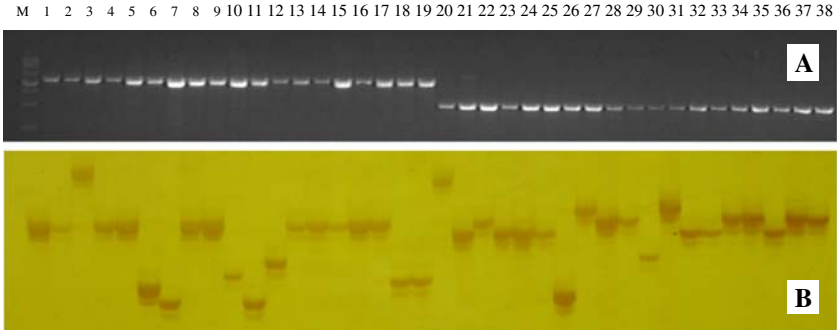

Fig. 4 The polymorphism revealed by STS marker H90 (**a**) and SSR marker RM24481 (**b**) in 38 typical PE and drooping panicle varieties. *Lane 1–19*, erect panicle varieties (panicle curvature <30°); *lane 20–38*, drooping panicle varieties (panicle curvature >80°). *1*, Huajing No. 2; *2*, Wuyu 2105; *3*, Wuyujing 7; *4*, Huaidao 8; *5*, Nanjing 39; *6*, Wuxiangjing 8; *7*, Xinghua 2001-4; *8*, Suxiangjing 1; *9*, Siyang 1382; *10*, Liangjiajing 1; *11*, Suxiangjing 14; *12*, Zhendao 916; *13*, Wuyujing 8; *14*, 998–3; *15*, Zhendao 210; *16*, R254; *17*, IT111; *18*, Balilla; *19*, Guihuahuang; *20*, Baxihandao; *21*, Yangdao 4; *22*, Diantun 502; *23*, Yanjing 204; *24*, Yan 93538; *25*, Yueguang; *26*, Zaoshajing; *27*, Wu 99–2; *28*, Woaiga; *29*, Zhonghua 11; *30*, Nongken 57; *31*, Sidao 10; *32*, Xiaoqianyou; *33*, Huangjinqing; *34*, Xiushui 11; *35*, Nongyu 1898; *36*, Taibei 167; *37*, Dijin; *38*, Shuijin 3; *M*, 250 bp DNA ladder marker (Takara). A 3.0% agarose gel was used in the electrophoresis for H90, and the gel was subsequently stained with EtBr; a 6% polyacrylamide gel was used for RM24481, followed by silver staining

PE. The QTL was positioned 1.8 cM from the STS marker H90 and accounted for 41.72% of the phenotypic variation in the DH population, further confirming the PC trait was governed by one major QTL, in agreement with the findings by previous studies (Xu et al. 1995a, b; Wang et al. 1997; Kong et al. 2007). The result of comparison of the location of *qPE9-1* with the *EP* gene identified by Kong et al. showed that, *qPE9-1* was 9.9 cM far from SSR marker RM257, and *EP* gene was 10.2 cM far from it, suggesting *qPE9-1* and EP gene may be same locus. Moreover, PE trait of both Wuyunjing 8 and Liaojing 5 was verified to originate from Balilla (Fig. 3), and both of PE parents had same genotype on H90 marker. Apart from *qPE9-1*, in present study, another QTL, *qPE9-2*, was also identified, accounting for about 26% total PC variation, which was not found in Kong's research. Hence, the two QTL, *qPE9*-1 and *qPE9-2* should be considered simultaneously in PE variety development in the future. In fact, the PE original parent Balilla is a semi-erect variety, not a typical PE one, however, Wuyunjing 8 and Liaojing 5 were typical erect panicle varieties, suggesting that some other genes must exist to work combined with *qPE9-1*. The availability of closely linked markers identified in the present study would potentially facilitate the marker assisted selection of this trait in rice breeding.

Many studies observed the relationship between PC and other traits (Jin et al. 2003; Xu et al. 1995b, 2005a, b; Wang et al. 1997). Xu et al. (1995b) reported that PC correlated positively to PH while data by Wang et al. (1997) suggested PC was positively correlated to PH, PL, NPB and filled grains per panicle, and negatively correlated to density of grains. The present study is the first to report the characterization of *qPE9-1*, mainly responsible for PC variation. Our results showed that in the DH population, PC correlated to PH, PL and NGPB. Additionally, QTL mapping data further indicated that the QTLs, *qPE9-1*, *qPL9* and *qPH9-1*, responsible for PC, PL and PH, respectively, were located in the same region on chromosome 9. These data suggested a major gene, residing in this region, might be responsible for all these three traits, implying the pleiotropic nature of the gene. Although *qPE9-1* has a negative effect on PH and PL, subsequently resulting in the yield decreasing, most of PE varieties now cultivated have high yield level. The possible reason is deduced that, some genes with positive effect on PH and PL were introduced and functioned with *qPE9-1* during the process of PE variety development.

The availability of a large number of molecular markers has facilitated the understanding of the genetic mechanisms for various traits in rice. Panicle traits of rice have been extensively studied, due to their association with rice yield

(Li et al. 2001; Brondani et al. 2002; Xing et al. 2002; Yamagishi et al. 2002; Kobayashi et al. 2004; Ashikari et al. 2005; Mei et al. 2006; Tian et al. 2005, 2006; Yoon et al. 2006). Brondani et al. (2002) studied 11 agronomic traits in BC2F2 families of the interspecific cross *Oryza sativa* × *O. glumaepatula* in two locations; four QTLs for PL were detected residing on chromosomes 4, 5, 7, 8 and 11, respectively. Mei et al. (2006) identified seven QTLs for PL located on chromosomes 1, 2, 5, 6, 7 and 8 in both recombinant inbreed lines from varieties *Lemont/Teqing* and two backcross hybrid (BCF1) populations derived from RILs, with QTLs in both populations explaining 10.3 and 26.8% of total variation, respectively. Research of an advanced backcross populations between *O. grandiglumis* and *O. sativa japonica* cultivar Hwaseongbye suggested one QTL on chromosome 6 was associated with PL (Yoon et al. 2006). Although several QTLs have been identified to be associated with PL, to date, none have been reported on chromosome 9. This further indicated that the *qPL9* on chromosome 9 with pleiotropic effect on PH (*qPH9)* and PC (*qPE9-1*) traits was a novel gene, exhibiting its greater value in breeding PE *japonica* varieties.

The history of rice varieties with PE in China can be traced as far back as 1964. From the progeny of natural hybrids of Balilla, a PE variety Suzhou 63-2 was successfully developed by Taihu Institute of Agricultural Sciences in Jiangsu Province. A well-known PE variety, Guihuahuang, developed from the progeny of Suzhou 63-2, was then released. In Liaoning Province, the first PE variety (Qianchonglang) was developed by crossing Balilla with other *indica* and *japonica* varieties in 1974. Since then, 30 varieties have been released in the Liaoning province, 20 of which were bred through selections from the progeny of a semi-erect panicle variety Balilla, including Liaojing 5, the most popular PE variety (Zhang et al. 2002a, b). This suggests that the genes associated with PE trait in most PE varieties currently cultivated in China are from Balilla. However, it has also been suggested that PE lines could be obtained by crossing *indica* with *japonica* varieties. For example, some PE lines segregated out from the progeny of a cross between *japonica* variety Zhenxiqiuguang and *indica* variety Yangdao 6 (93-11) (Tang, unpublished), suggesting that genes associated with PC may be divergent in rice. In the present study, the *qPE9-1* was identified to be responsible for 41.72% of PC variation, acting as a main genetic factor controlling PC trait. Moreover, PC was associated with many other agronomic traits (Table 2, 3), it is inferred that some other genes must exist to be responsible for PE phenotype. Therefore, research is still needed to explore other possible genes interacting with *qPE9-1*.

Little research has been focused on PC inheritance until recently when the PE trait gradually became an important character in rice breeding program. However, associated traits, such as the dense panicle, were characterized some decades ago, with the identification of three genes, *Dn*1 (Nagao and Takahashi 1963), *Dn*2 (Jones 1952) and *dn*3 (Futsuhara and Kitano 1979). Plants with the *Dn1* gene exhibited a greater number of secondary branches per primary branch and spikelets per panicle, as well as a decrease in PL, primary and secondary branches. While *Dn*2 phenotype showed compact, spike-like panicles with plumper seeds and short, stiff, relatively thick culms; *dn3* was associated with a large number of spikelets and panicle branches, shorter panicles and fewer primary branches, as well as malformed floral organs, laminated palea and/or lemma, depressed palea and/or lemma, and long, empty glumes and spikelets with adventitious roots frequently noted even under normal conditions. While *Dn*1 was assigned to chromosome 9, similar to *qPE9-1* in the current study; *Dn*2 and *dn*3 have not been mapped. In addition, a short panicle gene, *sp*, on chromosome 11, was characterized, and was associated with the absence of primary branches in the lower part of the ear axis (Iwata and Omura 1971). Additional research is needed to elucidate the relationship between *Dn*1 and *qPE9-1* due to their similar chromosome position and phenotype.

In the current study, the DH population, consisting of 154 lines, was derived from the cross between two *japonica* varieties Wuyunjing 8 and Nongken 57 with contrasting PC traits. Most lines used for QTL mapping exhibited >90% SS, enhancing accurate trait measurement. Additionally, a rare polymorphism was observed between the parents used in this study; of the 100 STS markers around *qPE9-1*, only eight were polymorphic between two parents, based on the sequence diversity between *indica* rice 93-11 and *japonica* rice Nipponbare. The low rate of polymorphism between Wuyunjing 8 and Nongken 57 made it difficult to saturate the molecular map and further clone the QTL utilizing a map-based cloning strategy. However, the co-segregation marker H90 among erect and drooping panicle cultivars may facilitate future map-based cloning research and serve as a useful tool in marker-assisted selection in breeding PE rice, and facilitate future cloning of the *qPE9-1* gene as well.

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