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ORIGINAL PAPER

Quantitative trait locus analysis and fine mapping of the *qPL6* locus for panicle length in rice

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Abstract

Key message **Two QTLs were identified to control panicle length in rice backcross lines, and one QTL *qPL6* was finely mapped with potential in high yield breeding.**

Abstract Panicle length (PL) is the key determinant of panicle architecture in rice, and strongly affects yield components, such as grain number per panicle. However, this trait has not been well studied genetically nor its contribution to yield improvement. In this study, we performed quantitative trait locus (QTL) analysis for PL in four backcross populations derived from the cross of Nipponbare (*japonica*) and WS3 (*indica*), a new plant type (NPT) variety. Two QTLs were identified on chromosome 6 and 8, designated as *qPL6* and *qPL8*, respectively. Near-isogenic lines (NILs) were developed to evaluate their contribution to important agronomic traits. We found that *qPL6* and *qPL8* had additive effects on PL trait. For the *qPL6* locus, the WS3 allele also increased panicle primary and secondary branches and grain number per panicle. Moreover, this allele conferred wide and strong culms, a character of lodging resistance. By analyzing key recombinants in two steps, the *qPL6* locus was finely mapped to a 25-kb interval, and 3 candidate genes were identified. According to the single nucleotide polymorphisms (SNPs) within candidate genes, 5 dCaps markers were designed and used to get haplotypes of 96 modern Chinese varieties, which proved that *qPL6* locus is differentiated between *indica* and *temperate japonica* varieties. Taken together, the superior *qPL6* allele can be applied in rice breeding programs for large sink size, particularly for *japonica* varieties that originally lack the allele.

Introduction

Rice (*Oryza sativa* L.) is an important staple crop that feeds about one half of the world population, and rice yield improvement is the primary breeding goal to satisfy demands of the ever-increasing population. There are four components that make up the rice yield, i.e., panicle number per plant, spikelet number per panicle, seed setting rate and grain weight (Xing and Zhang 2010). In the process of exploring yield components, panicle length (PL) is usually measured as the yield-related trait; however, genetic factors of PL and their effects on rice yield improvement have not been well recognized (Hittalmani et al. 2002, 2003; Thomson et al. 2003; Cho et al. 2007; Marathi et al. 2012). On the other hand, it has been reported that PL trait is positively associated with rice yield, and can be used as selection criteria for yield breeding (Li et al. 2011; Yadav et al. 2011). In general, a rice panicle consists of one rachis and ten or more primary branches that bear several secondary branches, and PL is determined by the rachis length plus

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primary branch length above rachis tip, which varies widely among cultivars (Ikeda et al. 2004). Like other panicle elements, PL is inherited in a quantitative manner and controlled by both major and minor QTLs (Liu et al. 2011a). Moreover, genotype by genotype (G × G) interaction and genotype by environment (G × E) interaction should have contribution to the trait variation (Xing et al. 2002).

The advent of saturated molecular makers and analytical tools has facilitated the investigation of genetic bases of quantitative traits. In the past two decades, a number of QTLs for PL and other yield-related traits have been identified on the 12 chromosomes in rice, suggesting that PL is controlled by multiple factors (Xiao et al. 1998; Hittalmani et al. 2002, 2003; Thomson et al. 2003; Kobayashi et al. 2003; Lee et al. 2005; Mei et al. 2005; Cho et al. 2007; Liu et al. 2011a; Marathi et al. 2012). Interestingly, many beneficial alleles for long PL are from wild rice, making it the useful source for rice yield improvement (Xiao et al. 1998; Lee et al. 2005). Cultivated rice was originated from wild rice and two subspecies groups (*indica* and *japonica*) have been evolved by multiple domestications, which harbor genetically distinct gene pools for various traits (Khush 1997; Kovach et al. 2007; Huang et al. 2012). The favorable alleles for yield-related traits are distributed between the two groups, and introgression or pyramiding of these alleles can break the genetic barrier of yield improvement (Marathi et al. 2012). However, some alleles including those for PL are sensitive to growth environments. For example, Hittalmani et al. (2003) identified eight QTLs for PL at nine locations using a double-haploid (DH) population, but only two of them were consistently detected at all the locations. Similar results were obtained from the analysis performed over 3 years at two locations, and only loci detected in more than two environments were categorized as main effect QTLs (Cho et al. 2007). Therefore, QTLs that function consistently over a range of environments are preferred for plant breeding, but application of environment-sensitive QTLs to specific locations could be an alternative.

Correlation analysis has revealed significant relationships between PL and other agronomic traits, including culm length, tiller number, panicle exsertion, third node width, secondary branch number and spikelet number per panicle, and these correlated traits were usually mapped synchronously (Xiao et al. 1998; Thomson et al. 2003; Lee et al. 2005; Cho et al. 2007; Marathi et al. 2012). Moreover, the study for genetic dissection of rice plant architecture showed that the QTLs for PL were co-localized with either culm length or tiller number or both of them (Kobayashi et al. 2003). However, it is unclear whether they are in tight linkage or due to pleiotropy of the genes involved. It has been reported that the PL trait was co-segregated and finely mapped with the same locus for spikelet number per panicle, demonstrating the pleiotropic effects of the underlying genes (Tian et al. 2006; Xie et al. 2008). The identification and mechanistic explanation of these PL genes would pave the way for further yield exploration in rice, and several genes have been recently investigated. The *dep1* locus is a gain-of-function mutation causing truncation of a phosphatidylethanolamine-binding protein-like domain protein. The mutant allele results in reduced length of inflorescence internodes/PL but increased number of grains per panicle and consequently increases grain yield (Huang et al. 2009). *Short panicle1* (*SP1*) is a mutant defective in rice panicle elongation and thus leads to short panicle phenotype. *SP1* encodes a putative transporter that belongs to the peptide transporter, and consistent with the aberrant panicle phenotype, the gene is highly expressed in the phloem of young panicle (Li et al. 2009). In this study, we performed QTL analysis for PL using advanced backcross populations by scanning the residual heterozygous regions, reminiscent of principle of heterogeneous inbred family (HIF) analysis. Two PL loci *qPL6* and *qPL8* were detected at different locations and NILs were developed to validate their genetic effects. Fine mapping was performed to identify the underlying gene of *qPL6* for its potential in increasing sink size, and the allele distribution was analyzed among modern varieties by SNP markers in the candidate genes, facilitating the application of the beneficial allele in rice breeding.

Materials and methods

Plant materials and population development

Backcross lines with PL segregation were identified from the BC_3F_3 generation derived from the cross of recurrent parent Nipponbare (*japonica*) with small panicle and narrow stem and donor parent WS3 (*indica*), a traditional NPT variety with large panicle and wide stem. To clarify the genetic bases of PL in WS3, four segregating populations (BIL24, BIL26, BIL27 and BIL28) with 77, 133, 94 and 93 individuals were planted at the Lingshui experimental station in the Hainan Island for QTL analysis in 2010. To confirm the QTL performance, BC_3F_4 lines BIL28-18 with heterozygous QTL regions were planted at the experimental station in Shanghai for independent analysis in 2011. By genotyping progenies of a single plant from BIL28-18 (BIL28-18-92), four BC_3F_6 plants with fixed genomic background were selected as NILs, which contained all the homozygous allele combinations of the two detected QTLs, and the NILs were grown for trait assay in Shanghai both in 2012 and 2013. To finely map the QTL, recombinants from heterozygous QTL region were identified in two steps. Firstly, three primary recombinants (R1-1, R1-2 and R1-3)

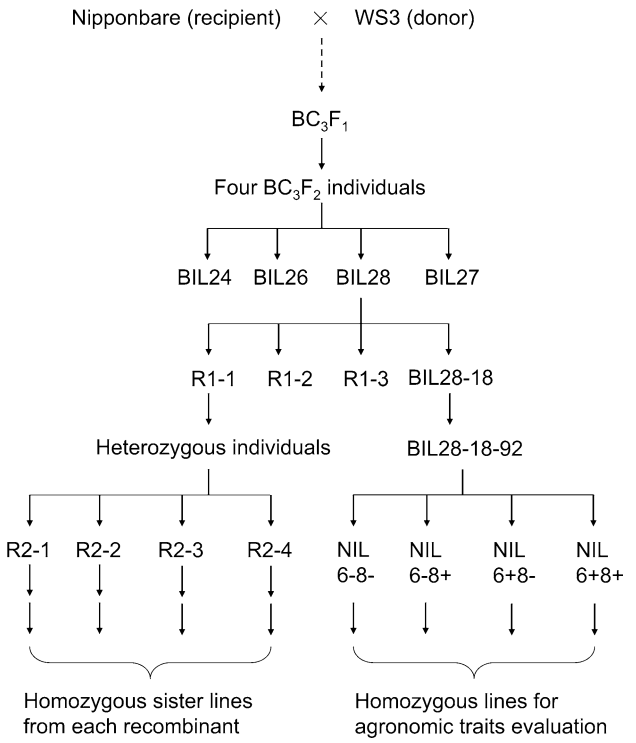


Fig. 1 The sketch map of materials' development for QTL analysis, NIL trait comparison and QTL fine mapping. *Dotted arrow* denotes backcrossing three times, and each *solid arrow* denotes selfing one time

were selected from BIL28 and their selfing populations with 110, 114 and 56 individuals were planted for progeny analysis to determine the phenotype explicitly and narrow down the QTL region. Then, new recombinants within the region (R2-1, R2-2, R2-3 and R2-4) were selected from progenies of heterozygous plant (from R1-1) by flanking markers, and their selfing progenies were further genotyped and reciprocal homozygous siblings were grown with two repeats for PL comparison under paddy field conditions. The procedure for the materials' development is illustrated in Fig. 1 to make it clear. In addition, 48 *indica* and 48 *temperate japonica* modern varieties were planted and used to detect the allele distribution of the target QTL (*qPL6*) (Table S1).

Measurement of agronomic traits

PL was measured as the length from the panicle neck to panicle tip of the main panicle, and data were subjected to QTL analysis, NIL comparison and gene fine mapping. To find the relationships between PL and other agronomic traits, plant height (PH), tillers number (TN), primary branch number (PBN), secondary branch number (SBN), spikelet number per panicle (SPP), seed setting rate (SR) and stem diameter of the third internodes (SD) were also evaluated among NILs. PH was measured as distance from ground to tip of the main panicle. TN was scored as the number of total reproductive tillers of a whole plant at mature stage, and PBN and SBN were scored as the number of primary and secondary branches from the main panicle. SPP was obtained by dividing total spikelet number by TN and SR was the result of dividing total grain number by total spikelet number per plant in 2012. Data for SPP and SR in 2013 were obtained from the main panicle. SD was measured by the slide caliper from the major axis and minor axis of main culms after removing the leaf sheath, and the mean of two values was used for analysis.

Marker development and genotype analysis

DNA was extracted from leaves for PCR amplification following the previous methods (Xu et al. 2004). According to gramene database (www.gramene.org), genome-wide SSR markers with polymorphisms between the two parents were selected to search heterozygous regions of the populations. For the regions with no available polymorphic SSR markers, Indel (Insert or deletion) markers were designed by Primer Premier 5.0 based on the published rice DNA polymorphism database (Shen et al. 2004). In all, 276 polymorphic markers were applied evenly throughout the 12 chromosomes. The markers located in the heterozygous region were further used for QTL analysis of each population (Table S2). During QTL fine mapping, 8 new Indel markers were developed in the target region to determine recombination sites and genotype of recombinant progenies (Table 1). In addition, 5 dCaps markers were developed by dCaps finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html) according to the SNP information within candidate genes in the mapping region (Table 2), which were used to analyze haplotypes of modern rice varieties. For all types of markers, the reaction mixture (20 μL) for the PCR analysis consisted of 20 ng of template DNA, 2.0 μL of 10× PCR buffer, 2 μL of 2.5 mM dNTPs, 1 μL each of 5 μM primers and 0.5 U of *Taq* DNA polymerase. The thermal cycling consisted of initial denaturation at 95 °C for 5 min, followed by 36 cycles of denaturation at 95 °C for 30 s, annealing at primer-specific temperature for 30 s and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR-amplified products were mobilized by agarose gel (3.0 %) electrophoresis, and the gels were photographed under UV light. For dCaps markers, 10 μL PCR product was used for enzymatic digestion following the instruction of relative enzyme (Thermo Scientific), and then electrophoresis was performed.

QTL mapping and statistical analysis

The linkage map was constructed by the Mapmaker 3.0 based on the genotype data of each population, and

Table 1 Indel primers used in fine mapping of *qPL6*

Marker	Forward primers	Reverse primers
qPL6ID-1	GGAAAAACAATAGTAGTGTGGTG	TTATAATCCGAACAGAAAAATGA
qPL6ID-2	TAGTGAAAAAAGAAGAAACGATT	GAGTAGACCATGGCATATTAAGG
qPL6ID-3	TTAGTTGACTGAACCAAATAGCA	TGTTTAGAAGATCAAGGAGAGTG
qPL6ID-4	GCTCAAGTTTTAGCTTGTTT	AGTGATTTGTTAGTAGGGGT
qPL6ID-5	CAAAACACAGGAAAAACAC	TTAGTAAAAGCCCAAATCG
qPL6ID-6	AAGCTATACCAAGTACTCCCTCC	AAACAATGTCCGGTGTCTCTA
qPL6ID-7	AATCCGAAGATATTTTTTGAG	GGAGTAGAGTAGCAGTTGTTGA
qPL6ID-8	AGGAAATAATAGGGATGGTGC	TTTGAGTGAGAATCGTGCTG
InDel6-2	ACGGATAAGAATGGACGAAC	TAGAACCAGGGCAAGATGAC

Table 2 dCaps primers developed for SNP genotyping of modern varieties

Marker	Forward primers	Reverse primers	Enzyme	Genes	Position[a]
460dCaps-1	CGCCGGCTGCCGCAGCCGCTGGTGGACCGG	GGGGACGACGAAGGCGAAGA	BamHI	Os06g45460	115 (A-G)[b]
460dCaps-2	CCTGCCGGAGGCGAAGGCCATGCCGGGGC	CGCGTCGGGGCACAAGACGCTG	HaeIII	Os06g45460	676 (C-G)[b]
480dCaps-1	TGCTAATTCAGATAGCTTCATAATTGGAAT	TTGATATGGTCTGTACTGGTAAGT	HinfI	Os06g45480	434 (T-C)
480dCaps-2	CTTTTGCATAATTTTCGAGTAGCCTGCGGC	AATATCTGTGGGGTTCATGTGTGT	HaeIII	Os06g45480	653 (A-G)
480dCaps-3	TGCTTATGTAGAGAAGAACCTATACCTGAT	CTTGTCAGATACTTGTAAAATTGAT	HinfI	Os06g45480	1943 (T-G)

[a] Numbers indicate the position of SNP to ATG of the corresponding genes, and letters in parentheses are the nucleotide changes (Nipponbare vs WS3)

[b] SNPs that lead to amino acid change

Kosambi function was used to calculate genetic distances (Lander et al. 1987). WinQTLCart2.5 was employed for the detection of QTLs and composite interval mapping method was applied (Wang et al. 2012). The LOD threshold for QTL detection was determined by computing 1000 permutations (Churchill and Doerge 1994). One-way ANOVA method was applied to perform statistical analysis during gene fine mapping and NIL trait comparison, and Tukey's test was applied for multiple mean comparisons when necessary.

Results

QTL analysis of panicle length reveals two loci with additive effects

To dissect genetic control of panicle traits and provide novel genetic resource for yield breeding in rice, we crossed WS3 that bears large panicle with the model variety Nipponbare. In the BC_3F_3 generation, four lines (BIL24, BIL26, BIL27 and BIL28) were identified with PL segregation. All lines exhibited similar distribution pattern in a binomial way (Fig. 2), reflecting the simple genetic behavior of underlying loci. As only heterozygous regions lead to trait segregation, we first scanned the whole genome by molecular markers evenly distributed on the 12 chromosomes. The result showed that most of the genome had been substituted by the Nipponbare background, and small proportion of heterozygous regions and WS3 homozygous regions were retained (Fig. 3). With markers on heterozygous intervals (Table S2), the genotype of each population was obtained, and in combination with trait data, two QTL loci were repeatedly detected on chromosome 6 and 8, respectively, designated as *qPL6* and *qPL8* hereafter (Table 3). Although the two QTLs did not show high LOD values (from 3.09 to 7.01), each of them explained at least 13 % of total phenotypic variation, and particularly in line BIL26, two loci were detected and together explained 35 % of the total phenotype. The additive effect of the two loci was variable in different lines, from 1.08 cm to 1.81 cm. Moreover, the WS3 allele at *qPL6* contributed to PL increase, while the WS3 allele at *qPL8* decreased PL. QTL analysis in Shanghai also detected the two loci with similar phenotype contribution (Table 3), reflecting their stable genetic behaviors under different growth environments.

qPL6 confers better panicle architecture compared with *qPL8*

To evaluate the effect of *qPL6* and *qPL8* on panicle morphology thoroughly, we developed NILs with different

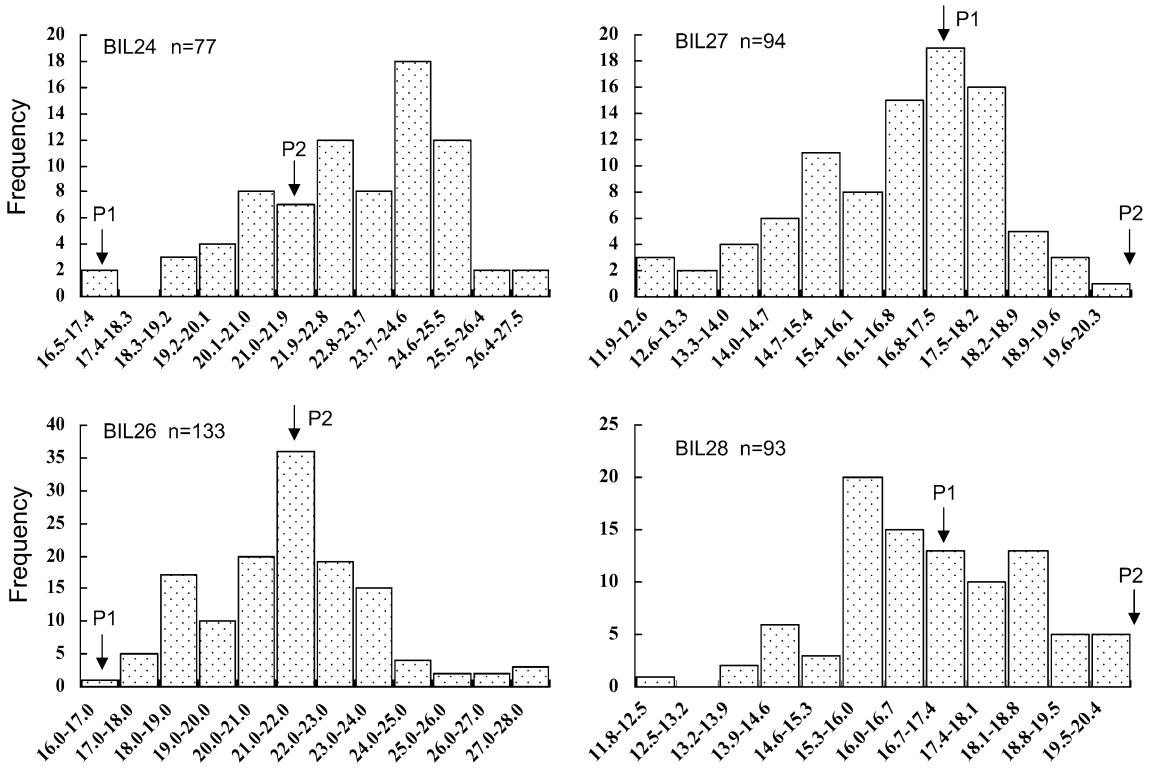


Fig. 2 Frequency distribution of panicle length in four backcrossed inbred lines. The numbers of individuals in each population were shown, and *arrows* with P1 and P2 indicate the position of Nipponbare and WS3 separately

combinations of the two loci and analyzed more agronomic traits in addition to PL. As shown in (Fig. 4a), no obvious difference could be found for the whole plant by visual observation. However, the difference of PL could be observed among the NILs and effect of *qPL6* was larger than that of *qPL8* (Fig. 4b). When two loci were pyramided, the trait performance for PL was better than lines with single locus (Fig. 4b). The statistical evaluation in 2 years showed similar trend for PL change (Fig. 4c, d), which further validated the existence of two loci and their stable genetic effect. Interestingly, we found that *qPL6* could increase panicle primary and secondary branches number in 2 years, resulting in production of much more spikelets per panicle, while the seed setting rate was not affected (Fig. 4c, d). Moreover, *qPL6* could also increase stem diameter, but had no obvious effect on plant height and tiller number (Fig. 4c, d), reflecting its potential in lodging resistance. As to *qPL8*, we did not observe significant effect on other traits (Fig. 4c, d), so it might only function in elongation of panicle.

Fine mapping of *qPL6*

As *qPL6* showed the great potential to increase yield, we focused on unveiling its underlying gene by map-based cloning strategy. According to the QTL confidence interval, the *qPL6* locus was located between markers InDel6-1 and InDel6-3 (Fig. 5a), and three recombinants in the QTL region were analyzed for primary mapping. The progenies of the three recombinants were genotyped and ANOVA analysis was performed to detect the phenotype difference among the three genotypes (Fig. 5b). Significant trait segregation was found in lines R1-1 and R1-2, which share a 163-kb heterozygous region flanked by markers qPL6ID-2 and qPL6ID-5, suggesting that the *qPL6* locus resides in this region. In agreement with this conclusion, R1-3 homozygous in this region did not show trait segregation. Therefore, the *qPL6* locus was primarily mapped to the 163-kb interval. Next, four more recombinants of this interval were identified by flanking markers (Fig. 5c). One recombination occurred between markers qPL6ID-6 and qPL6ID-7 in R2-1, while the other three recombinations occurred between markers qPL6ID-8 and InDel6-2 in R2-2, R2-3 and R2-4. Reciprocal homozygous progenies of the recombinants were planted for phenotype confirmation, and the result showed that PL of the progeny plants from R2-1, R2-3 and R2-4 but not from R2-2 were significantly different (Fig. 5c). Therefore, the *qPL6* locus was clearly narrowed down to a 25-kb region defined by markers qPL6ID-6 and InDel6-2.

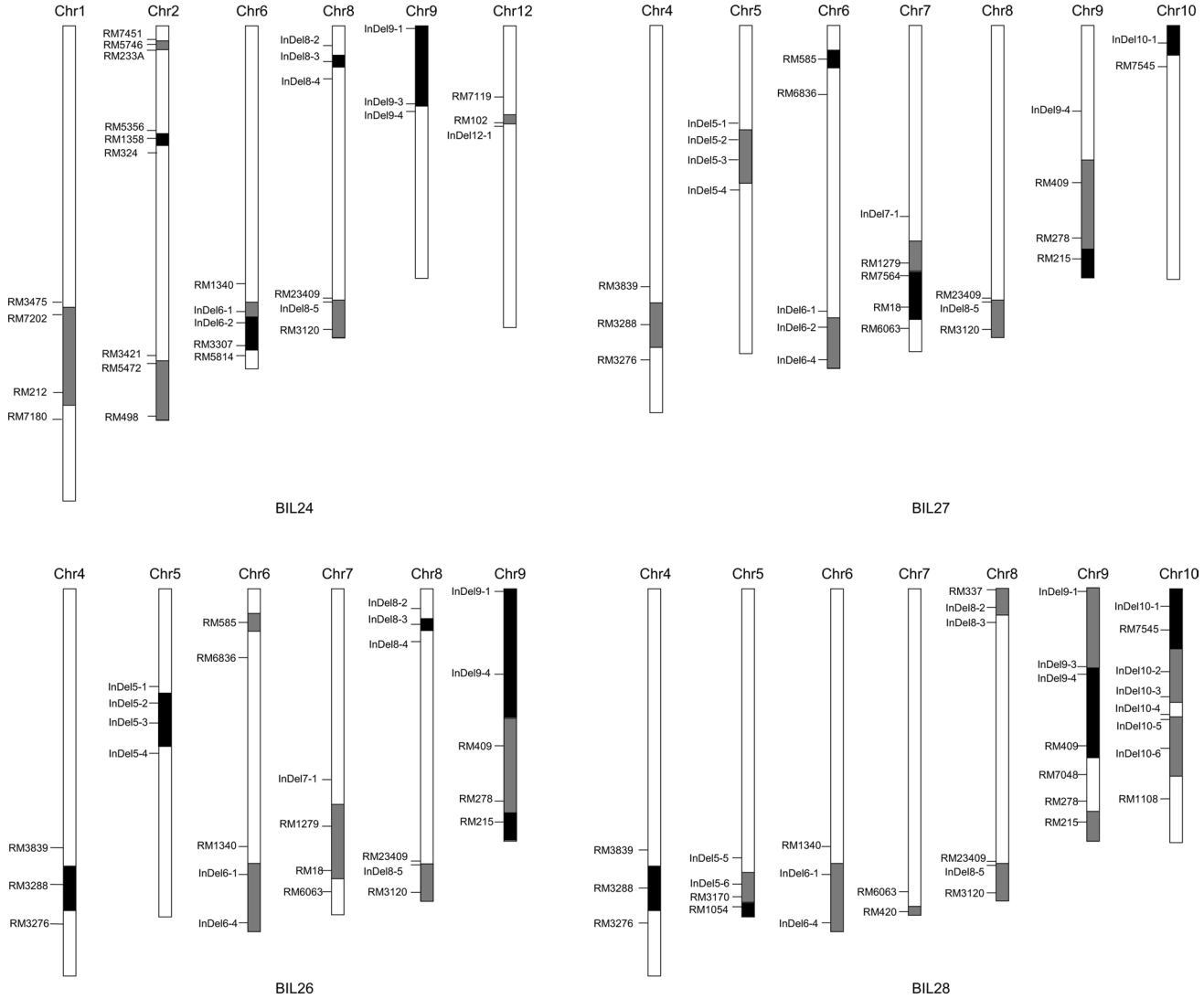


Fig. 3 Graphical genotype of four backcrossed lines. All the chromosome regions that include introgression from parent WS3 are shown. The *white* and *black bars* indicate the region of Nipponbare and WS3, respectively, and the *gray bars* indicate the heterozygous region. Markers that confine the borders of different regions are labeled

Table 3 QTL mapping in two locations

Families	QTL names	Chr	Peak position	LOD	Additive effect[a]	R^2 (%)[b]	Sites
BIL24	*qPL8*	8	RM23466	3.09	−1.81	22	Lingshui
BIL26	*qPL6*	6	InDel6-2	7.01	1.65	22	Lingshui
	qPL8	8	InDel8-7	3.57	−1.24	13	
BIL27	*qPL8*	8	RM23466	3.65	−1.12	20	Lingshui
BIL28	*qPL6*	6	InDel6-2	3.29	1.08	19	Lingshui
BIL28-18	*qPL6*	6	InDel6-2	6.12	0.78	20	Shanghai
	qPL8	8	InDel8-7	5.86	−0.85	18	

[a] Minus indicates that the promoting allele is from Nipponbare

[b] Percent of phenotypic variation explained by each QTL

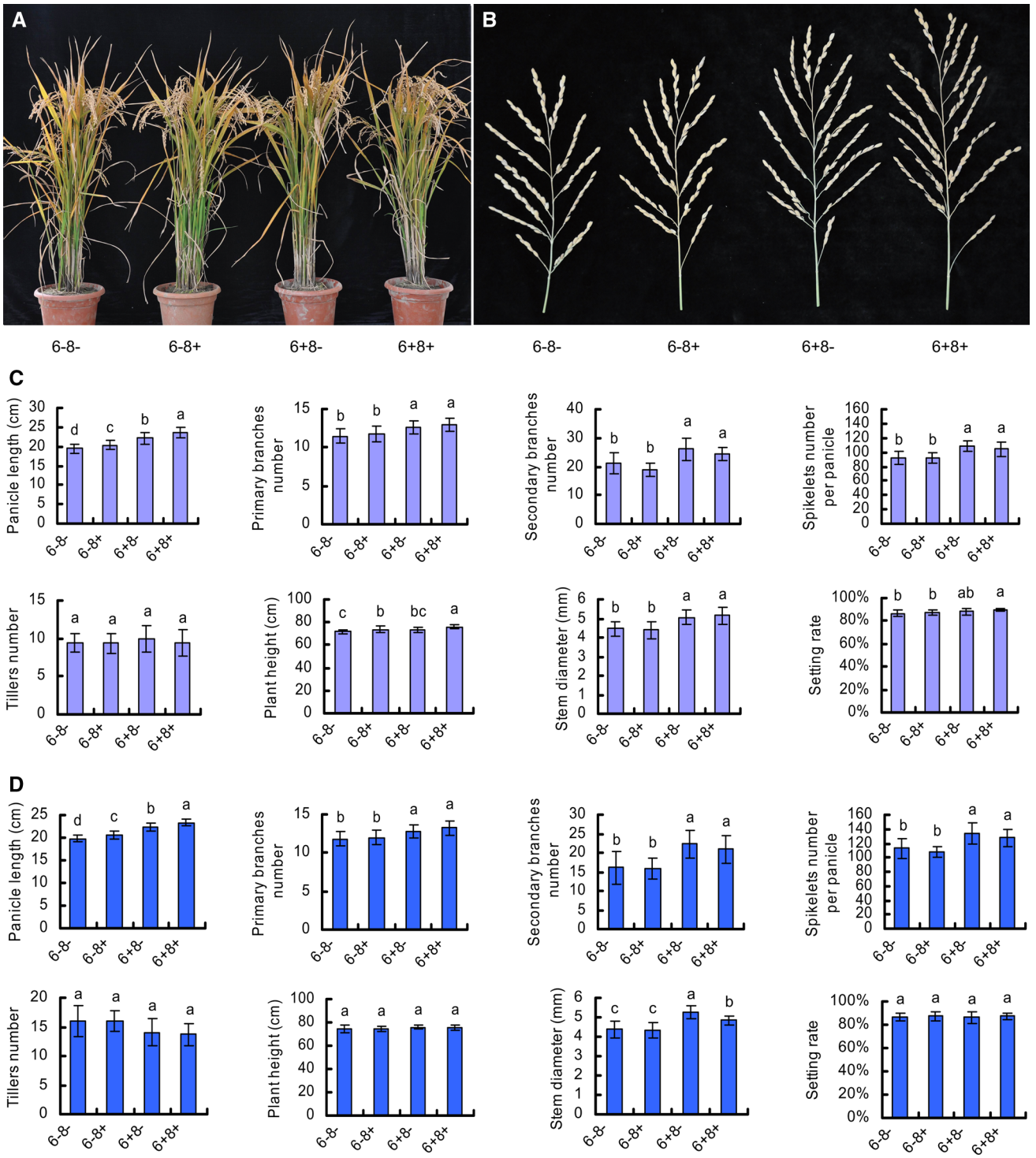
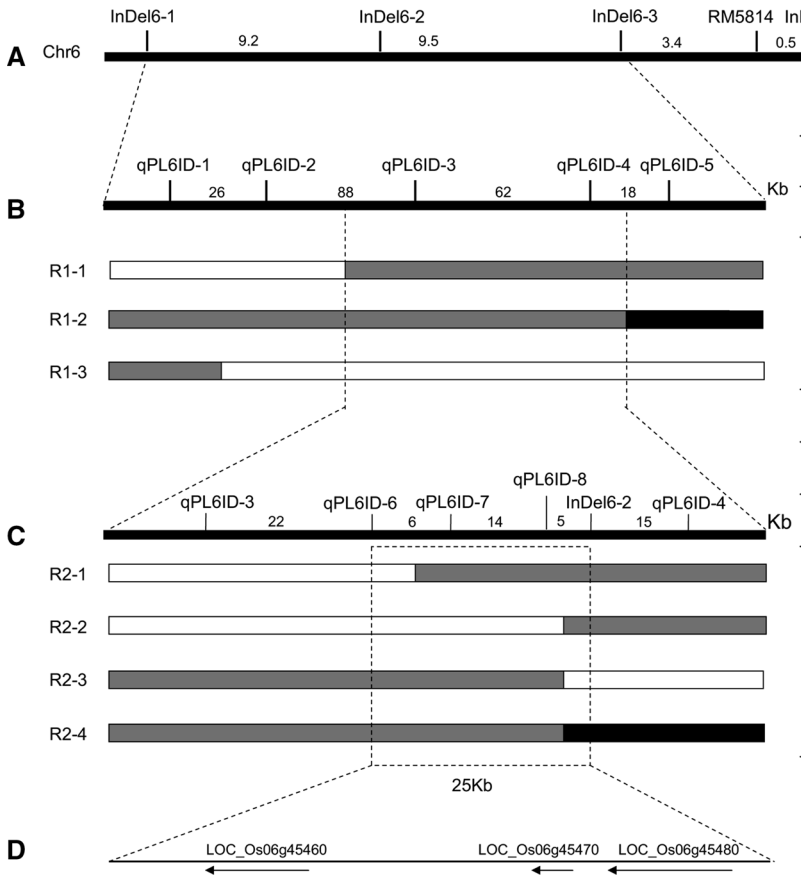


Fig. 4 Phenotyping of four NILs with allele combinations of *qPL6* and *qPL8* in 2 years. **a** The overall view of the four lines. *Numbers with plus and minus* indicate the existence or absence of superior alleles for two loci. **b** Panicle structure of four lines. **c** Trait comparison among four lines in 2012. Eight traits including panicle length (cm), primary branch number, secondary branch number, spikelet number per panicle, tiller number, plant height (cm), stem diameter (mm) and seed setting rate are shown by histograms. Bars followed by different letters are significantly different by multiple mean comparisons (Tukey's test, $n = 24$ for each genotype). **d** Trait comparison among four lines in 2013. Bars followed by different letters are significantly different by multiple mean comparisons (Tukey's test, $n = 12$ for each genotype)



	Mean ± SD(cm)			P-value
	WW	NN	WN	
R1-1	19.8 ± 1.28(25)	18.2 ± 0.75(19)	19.4 ± 1.10(66)	<0.0001
R1-2	19.9 ± 1.30(31)	18.6 ± 1.28(20)	19.7 ± 1.14(63)	0.0011
R1-3	17.8 ± 0.66(9)	17.6 ± 1.03(18)	17.6 ± 0.86(29)	0.91

	Mean ± SD(cm)			
	WW (L1)	NN (L1)	WW (L2)	NN (L2)
R2-1	23.0 ± 1.04[a]	21.8 ± 1.00[b]	22.9 ± 1.27[a]	21.5 ± 1.10[b]
R2-2	19.9 ± 0.67[a]	20.0 ± 0.64[a]	20.3 ± 0.56[a]	20.3 ± 0.51[a]
R2-3	20.9 ± 0.80[a]	19.6 ± 0.61[b]	21.1 ± 0.60[a]	20.1 ± 0.85[b]
R2-4	21.2 ± 0.87[a]	20.0 ± 0.83[b]	21.4 ± 0.82[a]	20.1 ± 0.82[b]

Fig. 5 Substitution mapping of *qPL6*. **a** The genetic linkage map of *qPL6* region on chromosome 6. Markers used are labeled on the *black bar*, and *numbers* between markers indicate genetic distance in centimorgans. **b** Primary mapping with three recombinants. The *black bar* with markers denotes the recombinant sites, and *numbers* between markers indicate the physical interval in kilobases. The *white*, *black* and *gray boxes* stand for genotypes of Nipponbare, WS3 and heterozygote, respectively. *Vertical dotted lines* denote the target region. Progeny test was performed by grouping the population into three genotypes with linked markers. WW, NN and WN are three genotypes representing WS3 and Nipponbare and heterozygote, and trait values are shown as mean ± standard deviation with individual number in *parenthesis*. *P* value indicates the significance of difference among genotypes by one-way ANOVA. **c** Fine mapping of *qPL6* with four recombinants. WW and NN are homozygous lines derived from the corresponding recombinants. L1 and L2 in *parenthesis* denote two repeated lines with the same genotype. Trait values are shown as mean ± standard deviation, and *different letters* indicate the significant difference by Tukey's multiple mean comparisons ($n = 16$ for each genotype). **d** Three candidate genes are identified in the fine mapping region. *Arrows* indicate direction of gene orientation

Candidate gene analysis of *qPL6* and haplotype survey among modern varieties

According to the rice genome annotation database (http://rice.plantbiology.msu.edu/), three genes were predicted in the mapping region, including *Os06g45460*, *Os06g45470* and *Os06g45480* (Fig. 5d). Through sequence comparison of the three genes in WS3 and Nipponbare, a number of sequence polymorphisms were found between the parents. However, only two SNPs and a 9-bp deletion in *Os06g45460* lead to amino acid change, and we thus proposed that *Os06g45460* might be the candidate for *qPL6*. Although, we could not exclude the possibility that other sequence polymorphisms might affect gene expression of the region and contribute to the PL phenotype, this needs to be further clarified by genetic complement experiments. To transform the sequence polymorphisms into molecular markers and use them for allele mining among modern varieties, five dCaps markers were developed according to two SNPs on *Os06g45460* and three SNPs on *Os06g45480* (Table 2). With these markers, 48 *indica* and 48 *temperate japonica* varieties were genotyped (Table S1). Interestingly, we found that the markers showed absolute linkage disequilibrium and exhibited strong differentiation between the two subspecies, which grouped all the *japonica* varieties to the Nipponbare genotype (J-type) and most *indica* varieties to the WS3 genotype (I-type) except four varieties (Table S1). This result suggests that the *qPL6* locus was originated independently in these *japonica* and *indica* accessions and the WS3 allele had not been introgressed into the *japonica*

genome during modern rice breeding in China. Therefore, the markers developed can be used for marker-assisted selection (MAS) to develop new variety with large panicle.

Discussion

Grain yield has long been one of the most important breeding targets in rice, and is controlled by many QTLs functioning in diverse biological processes. Identification of such QTLs and underlying genes will facilitate applying excellent alleles in rice yield improvement. In breeding practice, panicle length has been widely surveyed but attracts not much attention to clarify the underlying genes and relations to yield components. Here, we report the identification of two quantitative loci for panicle length, *qPL6* and *qPL8*, and fine mapping of *qPL6* to the 25-kb region. For QTL identification, we performed the analysis with advanced backcross generations at two locations. This strategy should enable us to identify the quantitative trait locus with relatively small effect, which could have been neglected in the primary population (Zhang et al. 2009). In addition, the purification of genetic background can reduce the possible intergenic interactions that mask the single gene effect (Yamamoto et al. 2000). Consequently, heterozygous lines with trait segregation from advanced population could be used effectively for QTL analysis. Indeed, the four lines we used contained multiple heterozygous regions, and two PL loci were clearly identified from these heterozygous regions.

On the other hand, development of NILs is a good strategy for QTL confirmation and evaluation of their genetic effect and also provides good materials for population development during QTL fine mapping (Ding et al. 2011). HIF is a fast strategy to develop NILs which depends on inbred line selfing and selection for unfixed regions among lines. If a target QTL resides in the unfixed region, NILs can be easily obtained from the progeny (Tuinstra et al. 1997). Such strategy has been successfully applied to QTL mapping in maize, rice and *Arabidopsis* (Loudet et al. 2005; Chung et al. 2011; Liu et al. 2011b). In our study, we developed NILs directly from the advanced population with different allele combinations at two loci and made sure the genetic background was fixed and similar. Consistent with the QTL result, NILs exhibited similar additive effect. It is interesting that the superior alleles could be contributed by both parents, i.e., *qPL6* from WS3 and *qPL8* from Nipponbare in this study. The introgression of superior *qPL6* allele into Nipponbare background produced the longest panicle among the allele combinations, making it valuable for breeding application. Importantly, *qPL6* has pleiotropic effect on other panicle morphology, including both primary and secondary branch number, as well as final grain number per panicle, which is the key determinant for rice yield. Moreover, *qPL6* also increased stem diameter, the key factor for resisting lodging (Kashiwagi et al. 2008). These characteristics together indicate that *qPL6* can be used as an excellent genetic resource in modern rice breeding for yield potential and lodging resistance. Nevertheless, the mechanism of pleiotropy should be ascertained by further investigation.

The previous studies have identified QTLs for PL on chromosome 6. However, the loci identified in three studies were located either on the short arm or the middle part (Mei et al. 2005; Cho et al. 2007; Liu et al. 2011a), and none is close to the *qPL6* locus. Kobayashi et al. (2003) identified two PL loci (*R6-1* and *R6-2*) on chromosome 6, and the *R6-2* locus shares the same region with *qPL6*. However, it exhibited large effect on plant height which was not found for *qPL6*. Another locus *qPLT6-1* also shares the region with *qPL6*, which was identified with double-haploid (DH) lines from the cross of *indica* and *tropical japonica* variety (IR64 × Azucena), and the *indica* allele of *qPLT6-1* contributed to PL increase (Hittalmani et al. 2003), reminiscent of the effect of *qPL6*. Therefore, at least three loci for PL scatter on chromosome 6, and the relationships between *qPL6* and other loci need to be confirmed by fine mapping of other loci in corresponding populations. In our study, *qPL8* exhibited smaller effect compared to *qPL6* as revealed by NIL comparison. Interestingly, a QTL locus (*R8*) for PL was also identified on the long arm of chromosome 8, but it exhibited relatively large effect, and the beneficial allele was from *indica* (Kobayashi et al. 2003), different from our result that the *japonica* allele increased PL. Thus, the *R8* locus could be a different locus or a stronger allele of *qPL8*. Cho et al. (2007) reported a cluster of QTLs for PL on chromosome 8, and the locus *pl8.3* was flanked by markers RM80 and RZ70A, similar to the position of *qPL8*. In addition, *pl8.3* was identified as a minor QTL and the beneficial allele was also from *japonica* (Gihobyeo). We propose that *qPL8* and *pl8.3* might be the same locus, which can be finally unveiled by narrowing down the region further.

The *qPL6* locus was finally confined to a 25-kb interval with three candidate genes. Sequencing revealed rich sequence divergence between the two parents, making it hard to identify the causative variation. However, the gene *Os06g45460* with amino acid change is supposed to be the most likely candidate, and encodes an F-box domain containing protein. Such genes participate in cell cycle control and regulate cell division by the ubiquitin pathway (Craig and Tyers 1999; Jurado et al. 2008), making it possible that *Os06g45460* shares the similar function, and decides panicle length by regulating cell division. To explore the allele types among modern varieties, we developed five PCR-based SNP markers based on sequence variances within the

candidate genes (Table 2), which can facilitate the genotyping manipulation. Interestingly, alleles of *qPL6* locus were likely differentially distributed between *japonica* and *indica* subspecies, or the alleles were independently selected during *japonica* and *indica* domestication, given that all *japonica* varieties tested lack the allele. When introgressed into the *japonica* variety Nipponbare, *qPL6* greatly increased grain number per panicle with no negative effect on seed setting rate. This will probably confer larger panicle and then increased yield sink size in *temperate japonica* breeding, since *temperate japonica* varieties usually bear short and small panicles. Therefore, the identification of *qPL6* allele will facilitate rice molecular breeding for high yield potential, and markers developed in this study can be utilized effectively in MAS breeding.

Author contribution statement L. Zhang and Z. He conceived the research and designed the experiments. J. Wang developed the backcrossed lines for QTL analysis. J. Wang provided the modern varieties for genotype analysis. L. Zhang performed most of the experiment including QTL analysis, NIL evaluation and fine mapping. L. Wang, B. Ma, Y. Qi, L. Zeng, Q. Li participated in genotyping and phenotyping. Z. He oversaw the entire study.

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Conflict of interest The authors declare that they have no conflict of interest.

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