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Characterization of *qPL5*: a novel quantitative trait locus (QTL) that controls panicle length in rice (*Oryza sativa* L.)

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Abstract Panicle length (PL) is an important trait that determines panicle architecture and strongly affects grain yield and quality in rice. However, this trait has not been well characterized genetically, and its contribution to yield improvement is not well understood. Characterization of novel genes related to PL is of great significance for breeding high-yielding rice varieties. In our previous research, we identified *qPL5*, a quantitative trait locus for PL. In this study, we aimed to determine the exact position of *qPL5* in the rice genome and identify the candidate gene. Through substitution mapping, we mapped *qPL5* to a region of 21.86 kb flanked by the molecular marker loci STS5-99 and STS5-106 in which two candidate genes were predicted. By sequence analysis and relative expression analysis, *LOC-Os05g41230*, which putatively encodes a BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, was considered to be the most likely candidate gene for *qPL5*. In addition, we successfully developed a pair of near-isogenic lines (NILs) for *qPL5* in different genetic backgrounds to evaluate the genetic effects of *qPL5*. Agronomic trait analysis of the NILs indicated that *qPL5* positively contributes to plant height, grain number per panicle, panicle length, grain yield per plant, and flag leaf length, but it had no influence on heading date and grain-size-related traits. Therefore, *qPL5* and the markers tightly linked to it should be available for molecular breeding of high-yielding varieties.

Keywords Rice · Panicle length · *qPL5* · Gene mapping

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Introduction

Ensuring food security for an ever-increasing world population mostly depends on increasing grain yield in crop plants (Gupta et al. 2006). Rice (*Oryza sativa* L.) is an important staple crop consumed daily by a large percentage of the global population. However, the current pace of the yield increase in rice is

insufficient to meet the needs of a world population predicted to reach 10 billion in 30 years (Hickey et al. 2019). Breeding practice shows that the cultivation of rice varieties with high and stable yields offers the opportunity to satisfy that demand. At present, molecular design breeding is showing great potential for the selection of excellent varieties, and the most crucial requirement is to identify a subset of genes/quantitative trait loci (QTLs) that control grain yield-related traits and to elucidate the interactions and genetic effects among these genes/QTLs (Marathi et al. 2012; Tian et al. 2015).

Panicle architecture, one of the most important morphological traits in rice that determines grain yield, consists of various quantitative characters such as panicle length (PL) and the number of primary branches (PBN) and secondary branches (SBN) (Crowell et al. 2016). PL has been defined as an important yield-related agronomic trait, and the genetic factors that control PL have been widely studied. During the past decade, based on the identification of mutants, several genes for PL have been cloned. *SP1* encodes a possible peptide transporter that regulates the elongation of the branch resulting in shorter panicle length (Li et al. 2009). *DEP2* encodes a plant-specific protein with unknown functional domains that regulates the rapid elongation of the axis and thus controls panicle length (Li et al. 2010). *DEP3* is predicted to encode a patatin-like phospholipase A2 superfamily domain-containing protein that regulates the formation of vascular bundles, leading to changes in panicle length and grain size (Qiao et al. 2011). *LP* encodes a Kelch repeat-containing F-box protein that may be involved in modulating cytokinin contents in plant tissues, resulting in changes to panicle length; this indicates that the levels of some plant hormones, such as cytokinin, are tightly associated with PL regulation (Li et al. 2011). These results have broadened our understanding of the genetic mechanisms underlying PL regulation. However, mutations in these genes result in pleiotropic phenotypes, and almost all of them are unfavorable and impractical. Thus, it is necessary to mine novel genetic factors for PL in nature germplasm.

Like other grain yield-related traits, PL is also a canonical complex trait controlled by major and minor QTLs (Liu et al. 2011). At present, many QTLs for PL have been extensively characterized in rice, and at least 250 QTLs have been identified on the 12 chromosomes (http://www.gramene.org/). *LP1*, a major QTL for PL, was mapped to a small region of ~ 90 kb on chromosome 9 (Chr. 9) (Liu et al. 2016). *qPL6*, a QTL controlling PL, was fine-mapped to an interval of ~ 25 kb on Chr. 6 (Zhang et al. 2015). Low-resolution mapping delimited *PL6-5*, a QTL responsible for PL, to a ~ 1.3 Mb interval on Chr. 6 (Sun et al. 2017). *qPL8*, a novel QTL for PL, was mapped to a ~ 278 kb region on Chr. 8 (Zhang et al. 2021). Also, it is notable that several QTLs for PL have been cloned. *DEP1* encodes a phosphatidylethanolamine-binding protein-like domain protein that regulates meristematic activity, resulting in changes in PL and grain number (GN) (Huang et al. 2009). *IPA1*, a gene that encodes OsSPL14 (SOUAMOSA PROMOTER BINDING PROTEIN-LIKE 14), is regulated by the microRNA (miRNA) OsmiR156 in vivo, and the mutant allele of *IPA1* disrupts OsmiR156-directed regulation of OsSPL14, resulting in rice plants with an ideal plant architecture and with increased PL and enhanced grain yield (Jiao et al. 2010).

As mentioned above, although some genes/QTLs involved in PL regulation have been fine mapped or cloned, the underlying genetic mechanisms for PL regulation are unclear. Moreover, favorable alleles that could be directly employed in molecular marker-assisted selection of high-yielding varieties are still rare. It is therefore worthwhile to conduct a continued search for favorable alleles that are involved in the control of PL. In our previous study, a novel QTL controlling PL on Chr. 5, designated *qPL5*, was identified using a set of chromosomal segment substitution lines (CSSLs) (Xu et al. 2019). In that study, we performed fine-mapping of *qPL5* and predicted the candidate gene for *qPL5*. Also, the genetic effects of *qPL5* on the main agronomic traits were evaluated, and the utilization potential of *qPL5* in genetic improvement of yield potential was discussed. Thus, our research not only lays the foundation for cloning *qPL5*, but also provides a new gene source for high-yielding rice breeding programs.

Materials and methods

Plant materials and population development

In our previous study, a population of CSSLs (named N1-125) that carry one or several chromosomal

segments from the *indica* variety '9311' in the *japonica* cultivar 'Nipponbare' (NP) has been successfully developed and widely used for gene mapping (Gao et al. 2020; Xu et al. 2017; Zhang et al. 2011). To precisely map *qPL5*, N58, a CSSL that carries a single substituted segment covering the location of *qPL5,* was selected from the CSSL population to cross with the recipient parent NP in the Spring of 2017. From this we developed the F_2, F_3, and F_4 segregating populations. The substitution lines (SLs) with '9311'-type homozygous genotypes were obtained from the recombinants in the F_2 and F_3 populations, and these SLs were grown for phenotypic evaluation from 2018 to 2021, and were used for fine mapping of *qPL5*.

To evaluate the genetic effects of *qPL5,* we developed two near-isogenic lines (NILs) carrying the '9311' allele of *qPL5*. The NIL in the NP background, a curved panicle-type *japonica* variety, called NP-*qPL5*, was developed from the gene mapping population. Using molecular marker-assisted selection, we developed a NIL named KWY3-*qPL5* in the 'Kangwuyujing3' (KWY3) background, an erect panicle-type *japonica* variety, using N58 as the donor and KWY3 as recipient through constant backcrossing followed by self-pollination (Supplemental Figure S1).

Field planting

The F_2, F_3, and F_4 segregating populations were grown in the experimental fields of Yangzhou University (YZU) in Jiangsu and Hainan provinces of China during 2018–2020. The parental lines and the newly-developed SLs were planted in the experimental fields of YZU and Jiudian (JD) (Yangzhou, Jiangsu Province, China). The NILs were planted in a randomized block design with two replications in the experimental field of YZU. The parental lines and the SLs were planted in 4-row plots with 10 plants per row in 2019–2021. NP and NP-*qPL5*, KWY3, and KWY3-*qPL5*, were grown in 20-row plots with 15 plants per row in the summer of 2021. The growing density was 15 × 25 cm for a line X row.

Trait measurements

The main agronomic traits, such as PL, plant height (PH), heading date (Hd), tiller number per plant (TN), grain number per panicle (GN), PBN, SBN, thousand-grain weight (TGW), grain length (GL), grain width (GW), grain yield per plant (GYPP), and flag leaf length (FLL) were assessed. The method used for phenotypic evaluation was previously described (Wang et al. 2017; Xu et al. 2017; Zhang et al. 2015). Briefly, the panicle architecture related traits, such as PL, GN, PBN, and SBN were recorded from six main panicles that were defined as the panicle from the tallest tiller at maturity. To measure the PH, TN and FLL, we selected six plants from the middle of the row in each line for phenotypic evaluation. Hd is defined as the day from sowing to 50% panicle appearance. GYPP was measured as the mean grain weight from 50 individual plants. In addition, 100 fully filled grains harvested from at least 10 individuals were used for measurement of GL, GW, and TGW using the MICROTEK ScanMaker i800 flat-bed scanner.

DNA extraction, PCR amplification, and molecular marker development

Genomic DNA was isolated from fresh leaves harvested from individual rice plants using the CTAB method with slight modifications (Murray et al. 1980). PCR amplifications were performed in 20 µl reaction volumes containing 10 µl 2 × Taq Master Mix (Dye Plus) (Vazyme, Nanjing, China), 2 µl of 0.2 µmol/L primer, 20 ng of template DNA, and 6 µl ddH_2O. The PCR-amplified products were separated by agarose gel (3.0%) electrophoresis, and the agarose gels were photographed under UV light.

The novel InDel molecular markers (Supplemental Table 1) that were used in the mapping of *qPL5* were designed using Primer 6.0 software based on genomic sequence variations between the *indica* and *japonica* rice subspecies (http://www.ncbi.nlm.nih.gov/). The dCAPS markers were designed with the dCAPS Finder 2.0 program (http://helix.wustl.edu/dcaps/).

The genetic background of KWY3-*qPL5* was determined with the 40 k rice SNP-array, a whole-genome single nucleotide polymorphism (SNP) array with 40,000 SNP and InDel markers (Greenfafa, Wuhan). In brief, a DNA pool consisting of three KWY3-*qPL5* plants was constructed and genotyped.

Quantitative real-time (qRT)-PCR

qRT-PCR assays for characterizing the expression patterns of annotated genes were performed at different stages of panicle development in NP-qPL5 and NP. Developing panicles at four stages (≤0.5 cm, 1 cm, 2 cm, and 5 cm) were selected as one biological replicate from the panicle initiation stage to the mature pollen formation stage (Zhou et al. 2018). Total RNA was extracted from the different panicle developmental stages using the Total RNA Isolation Kit (Vazyme, Nanjing, China), and the RNA was reverse transcribed into cDNA using the Fast Quant RT Kit (with gDNase) (Novoptotein, Suzhou, China). The amplification conditions consisted of one cycle of 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, then 95 °C for 15 s and 60 °C for 1 min, with a melting curve from 60 °C to 95 °C. The expression of each gene was compared between NP and NP-*qPL5* using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2013). The names and sequences of the primers used are given in Supplemental Table 1.

QTL mapping and statistical analysis

The inclusive composite interval mapping (ICIM) method was performed to identify QTLs using ICIMapping 4.0 software with the aid of the genotypes and phenotypes of 234 F_2 plants, in which the genotype of the '9311'-type, NP-type, heterozygous type, and the absence of marker type were assigned the values 2, 0, 1, and − 1, respectively.

Data analysis

The analysis of variance (ANOVA) package in SPSS 15.0 (IBM) was used for statistical analyses. Significant differences were determined at the $p < 0.05$ and $p < 0.01$ levels.

Results

Genetics validation of qPL5

In our previous study, *qPL5* was preliminarily mapped to a ~ 2.71 Mb region on Chr. 5, and there are three CSSLs (N22, N57, and N58) that harbor *qPL5* (Xu et al. 2019). In order to validate *qPL5*, we performed map-based cloning. Re-sequencing data from the CSSL population showed that N58 carries only a single '9311' chromosomal segment on Chr. 5 (from 18,695,860 bp to 24,855,730 bp) encompassing *qPL5* in the NP genetic background (Fig. 1A and Supplemental Fig. 2A and B). Based on this information, STS5-1, STS5-6, and STS5-12, three Indel molecular markers that map to loci within this target region, were used to genotype N58, and the substitution segment in N58 was further confirmed (data not shown). ANOVA of PL between NP and N58 revealed a significant difference, with N58 having longer panicles than NP (Fig. 1B–D). Also, GN and PH were obviously increased in N58 compared with NP (Fig. 1B, E, and F). There was no significant difference in Hd between N58 and NP (Fig. 1B, G). These results indicate that N58 carries *qPL5* on the introduced chromosomal DNA segment from '9311'. Thus, N58 was selected to develop the segregating population for validating and fine mapping *qPL5*.

To confirm the location of *qPL5*, 234 individuals in the N58/NP F_2 population were randomly selected for genotyping using seven polymorphic markers that map to loci distributed in the *qPL5* mapping region, and PL in these plants was evaluated. Panicle length in this population showed a continuous distribution, indicating that PL is a complex trait (Fig. 2A). A major-effect QTL that explains 23.21% of the phenotypic variance for PL was detected and localized to a ~ 910-kb region flanked by the marker loci STS5-6 and STS5-10 (Fig. 2B), and this QTL should be *qPL5*. Moreover, the PLs of the heterozygous plants were significantly longer than in homozygous NP plants but shorter than in 9311 homozygous plants, indicating that *qPL5* is a partially dominant gene (Fig. 2C).

Substitution mapping of qPL5

To narrow down the mapped region containing *qPL5*, 3600 F_2 plants from the N58/NP population were genotyped with the STS5-6 and STS5-10 InDel markers. As a result, we identified 65 recombinant individuals at these two marker loci. Using an additional six polymorphic markers that map between the STS5-6 and STS5-10 loci, the 65 recombinants were divided into nine groups, from which nine types of SLs were obtained (I–IX) (Fig. 3A, B). Progeny testing of the SLs showed that plants in groups I–III and V–VII had

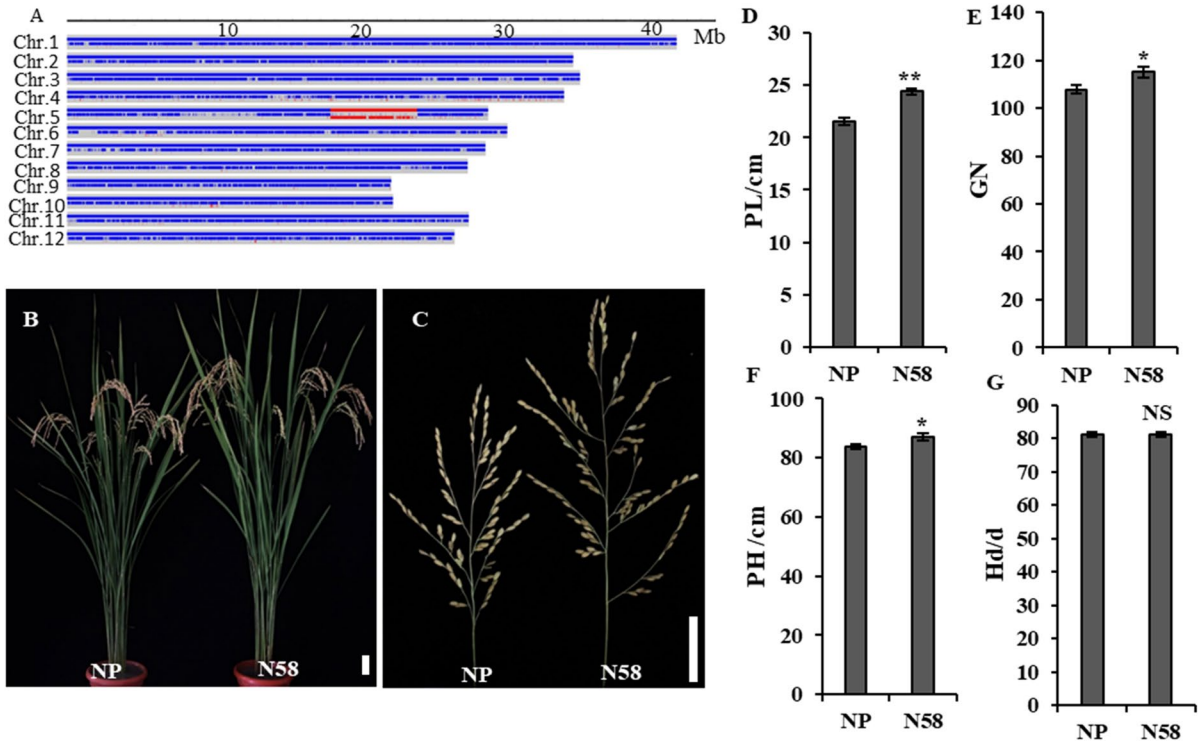


Fig. 1 Phenotypic differences between 'Nipponbare' (NP) and N58. **A** Graphical genotype of N58. The position of the introgressed chromosomal segment from '9311' is shown in red. **B** Mature plants of NP and N58. **C** Panicle phenotypes. **D**–**G** Comparisons of panicle length (PL), grain number per panicle (GN), heading date (Hd), and plant height (PH) between NP and N58. Error bars represent the mean value ± SE ($n = 6$ plants). ** and * indicate significant differences at $p < 0.01$ and $p < 0.05$, respectively, as determined by ANOVA. Scale bars = 5 cm in **B** and **C**

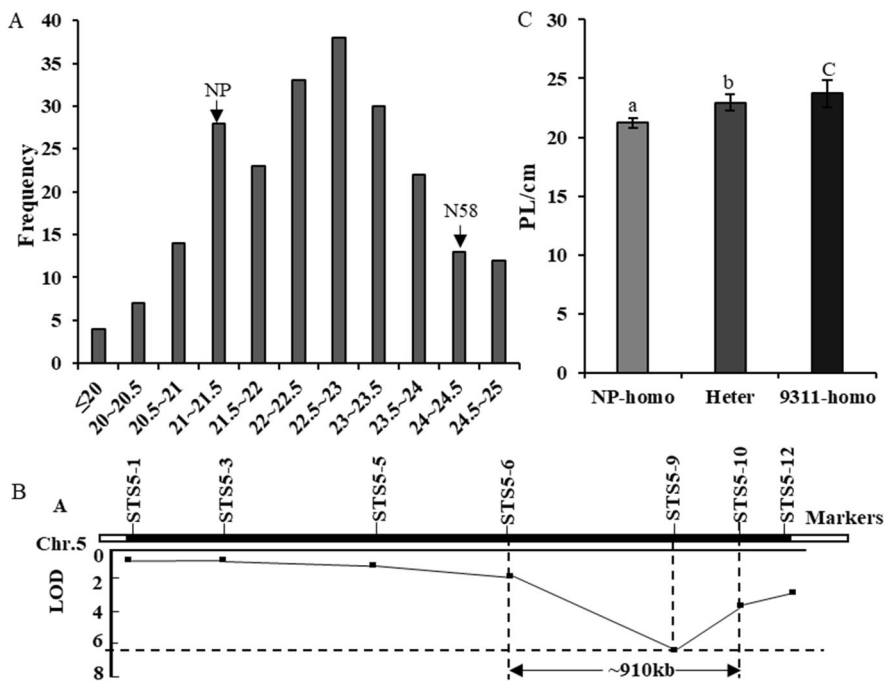
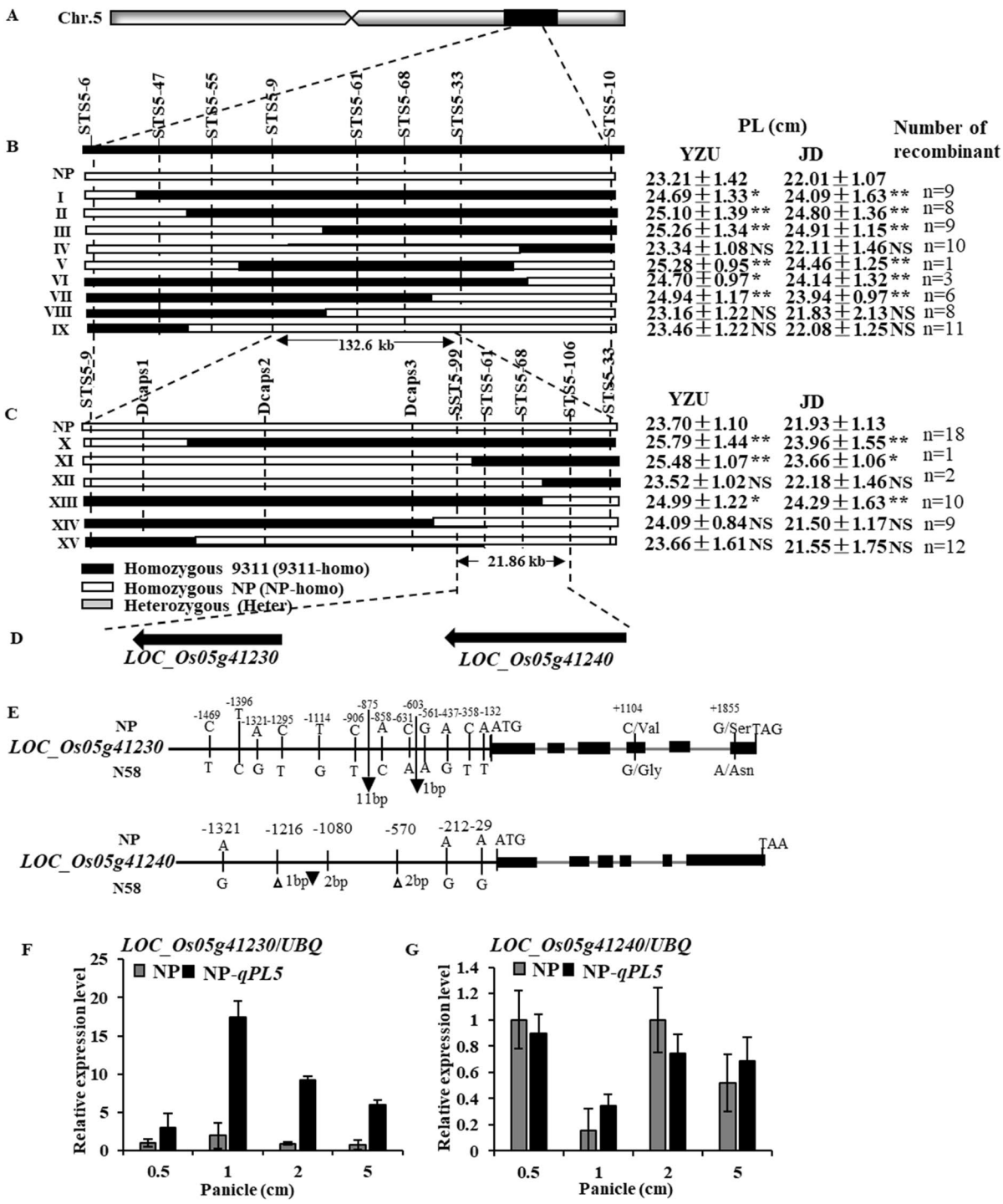


Fig. 2 Genetic validation of *qPL5*. **A** Frequency distribution of PL within the F_2 segregating population derived from NP and N58. **B** Localization of the QTL for PL detected in the F_2 segregating population to a region of ~ 910 kb on chromosome 5. **C** Comparison of PL between genotyped plants homozygous for the NP (NP-Homo) and '9311' ('9311'-Homo) alleles and the NP/'9311' heterozygote (Heter), which were selected from the F_2 segregating population. Error bars represent the mean value ± SE ($n = 40$ individuals). p values were calculated using one-way ANOVA



significantly increased PL compared with NP. In contrast, no significant differences in PL were observed between NP and plants in groups IV, VIII, and IX. Thus, we were able to reduce the *qPL5* interval to a genomic region of ~ 132.6 kb flanked by the STSN5-9 and STS5-33 marker loci (Fig. 3B).

To clarify the precise position of the *qPL5* locus, 15 individuals with heterozygous genotypes at *qPL5*

◂**Fig. 3** Fine mapping and candidate gene analysis of *qPL5*. **A** Physical location of the '9311' donor chromosomal segment on Chr. 5 in N58. **B**, **C** Primary mapping (**B**) and fine mapping (**C**) of *qPL5* based on the genotypes (left) and phenotypes (right) of the substitution lines (SLs). **D** Two open reading frames (ORFs) were identified within the 21.86-kb target region. **E** Comparison of the genomic sequences of the two ORFs from NP and N58. **F**, **G** Comparison of the expression levels of *LOC_05g41230* (**F**) and *LOC_05g41240* (**G**) in NP and NP-*qPL5*. Black and white rectangles represent homozygous '9311' and NP genotypes, respectively, in **A**–**C**. Δ and ▼ represent nucleotide deletions and insertions, respectively. Error bars represent the mean value ± SD ($n = 6$ individuals in each substitution line). Gene expression levels are presented as ratios of the expression of the individual ORFs to that of the rice *UBQ* gene. Means ± SD were obtained from three technical replicates and two biological replicates. ** and * indicate significant differences at $p < 0.01$ and $p < 0.05$, respectively, as determined by one-way ANOVA

in the F_2 population were selected to develop an F_3 population. Similarly, two molecular markers, STS5-9 and STS5-33, were initially used to identify the genotypes of 5,000 individuals, and a total of 52 recombinants were obtained at these two marker loci. Another seven polymorphic molecular markers that map between the STS5-9 and STS5-33 loci were then developed and used to detect the recombinants. We obtained six groups of recombinants, in which there were six types of SLs (X-XV). Progeny testing showed that plants of types X, XI, and XIII had longer panicles than did NP. In contrast, no significant differences in PL were observed between NP and types XII, XIV, and XV of the SLs (Fig. 3C). Finally, the location of *qPL5* was narrowed down to a genomic region of 21.86 kb that is flanked by molecular marker loci STS5-99 (24.147 Mb) and STS5-106 (24.168 Mb) based on the reference sequence of the NP genome (Fig. 3C).

Identification of the candidate gene for qPL5

According to the information of the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu), two predicted open reading frames (ORFs), LOC_Os05g41230 and LOC_Os05g41240, are present within this 21.86 kb interval. These genes are predicted to encode a BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor (BAK1) and a Myb-like DNA-binding domain containing protein, respectively (Fig. 3D). To identify the candidate gene for *qPL5*, the genomic sequences containing 2 kb of the promoter regions and the full coding regions of those two ORFs were sequenced to examine the sequence variation between the parental lines NP and N58. As a result, 12 base substitutions and two insertions in the promoter region, as well as two base substitutions in exons of LOC_Os05g41230, were identified between the two parental lines. Moreover, the variations in exon 4 and 6 were predicted to cause amino acid changes from valine to glycine and serine to asparagine, respectively (Fig. 3E). A sequence comparison of LOC_Os05g41240 identified six variations in the promoter region (Fig. 3E). Additionally, to analyze the relative expression of the two ORFs, NILs differing only in the *qPL5* region were developed in the NP genetic background, which carries a ~ 910-kb chromosomal segment introgressed from '9311' flanked by marker loci STS5-10 and STS5-6. The NILs carrying the '9311' and NP alleles in the *qPL5* region were designated as NP-*qPL5* and NP, respectively. The results showed that the mRNA levels of LOC_Os05g41230 were all higher in NP-*qPL5* than in NP at the different developmental stages of the young panicles. In contrast, the relative expression level of LOC_Os05g41240 did not show a significant difference between the two NILs (Fig. 3F). Taken together, LOC_Os05g41230 is predicated to be the most likely candidate gene for *qPL5*.

qPL5 increases panicle length and grain yield per plant

To clarify the genetic effects of *qPL5*, several agronomic traits in the NIL and receipt parent were evaluated. Like the phenotype of N58, PL in NP-*qPL5* was significantly longer (by 10.41%) than in NP (Fig. 4A, B, and E, Supplemental Table 2). We also found that SBN and GN were significantly increased in NP-*qPL5* compared with NP (Fig. 4F, G, Supplemental Table 2). The average increases in SBN and GN were 26.46% and 24.42%, respectively. Additionally, PH and FLL were both higher in NP-*qPL5* than in NP, with average increases of 3.97% and 9.13%, respectively (Fig. 4A, D, I, and J, Supplemental Table 2). There were no significant differences in Hd, PBN, and grain size-related traits such as GL, GW, and TGW, between NP-*qPL5* and NP (Fig. 4C, Supplemental Table 2). More importantly, compared with NP, GYPP in NP-*qPL5* was increased by 15.13%

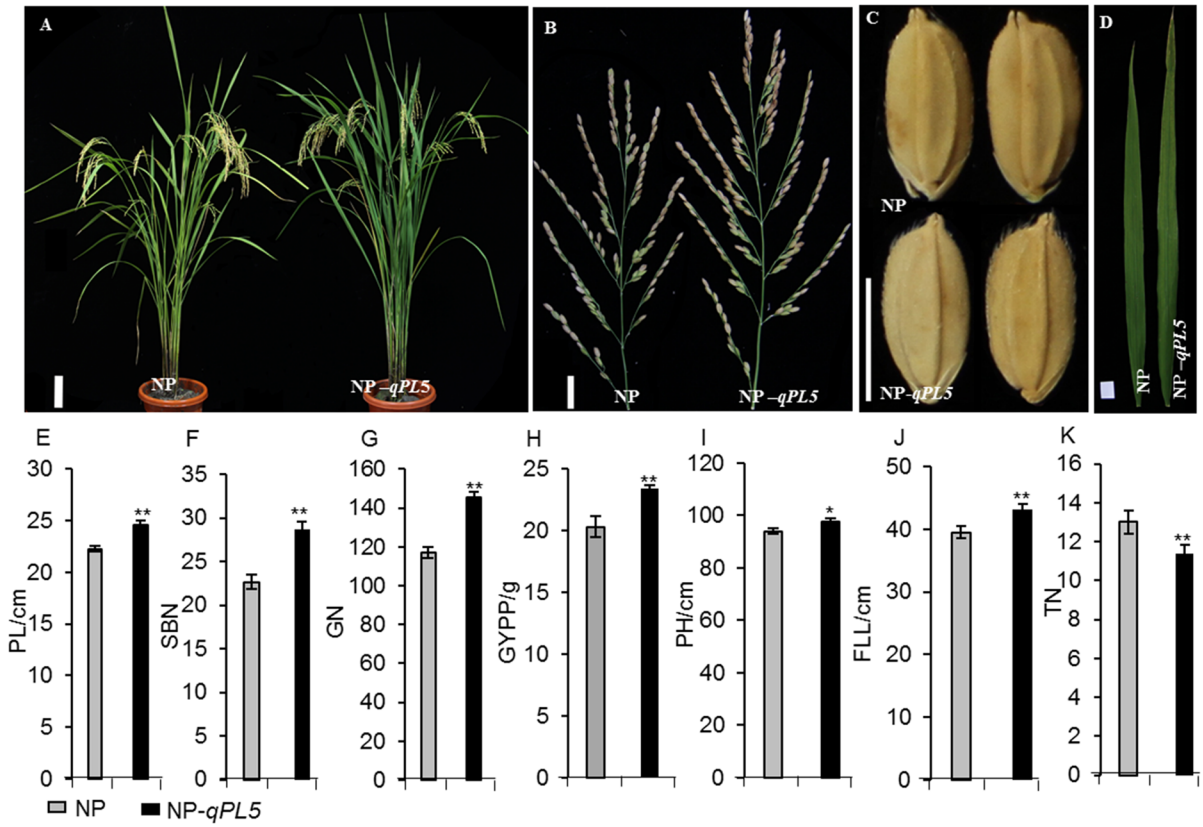


Fig. 4 Phenotypic characteristics of Nipponbare (NP) and the NIL, NP-*qPL5*. **A**–**D** Gross morphology of individuals at maturity (**A**), individual panicles (**B**), grain size (**C**) and flag leaf (**D**). Scale bars = 10 cm (**A**), 2 cm (**B**, **D**) and 0.5 cm (**C**). **E**–**K** Comparisons of PL (panicle length), SBN (secondary branch number), GN (grain number per panicle), GYYP (grain yield per plant), PH (plant height), FLL (flag leaf length), and TN (tiller number) between NP and NP-*qPL5*. Error bars represent the mean value ± SE of three biological replicates ($n = 50$ plants in H, $n = 6$ plants for the other traits). *P* values were calculated by one-way ANOVA. ** and * indicate significant differences at $P < 0.01$ and $P < 0.05$, respectively

although the TN was slightly decreased in NP-*qPL5* (Fig. 4H and K, Supplemental Table 2).

Considering that erect panicle architecture varieties are used widely in *japonica* production, we developed another NIL in the genetic background of KWY3, an elite erect-panicle architecture variety (Zhang et al. 2009). We obtained five BC_5F_3 lines (named HN1001-1005), and the genetic background of HN1001 was determined using the RICE 40 K SNP-array (Supplemental Fig. 1). The results showed that 97.60% of the KWY3 genome was recovered in HN1001, and only two introgression segments located on Chr. 5 and Chr. 11 were identified, one of contained *qPL5* (Supplemental Fig. 3). Thus, HN1001 could be considered to be a NIL for *qPL5* and was designated KWY3-*qPL5*. Compared with KWY3, KWY3-*qPL5* showed significant increases in PL, GN, SBN, PH, and FLL, while TN was slightly decreased (Fig. 5A–J, Supplemental Table 2). In addition, there were no significant differences in Hd, GL, GW, and TGW (Fig. 5A and Supplemental Table 2). Eventually, we found that GYPP in KWY3-*qPL5* was increased by 2.28% compared with KWY3 (Fig. 5G, Supplemental Table 2).

Overall, these data indicate that *qPL5* has a noticeable genetic effect on PL and most of the panicle-related traits but with no obvious impacts on grain size related traits and Hd, which shows that *qPL5* could be potentially useful in the breeding of high-yielding rice varieties.

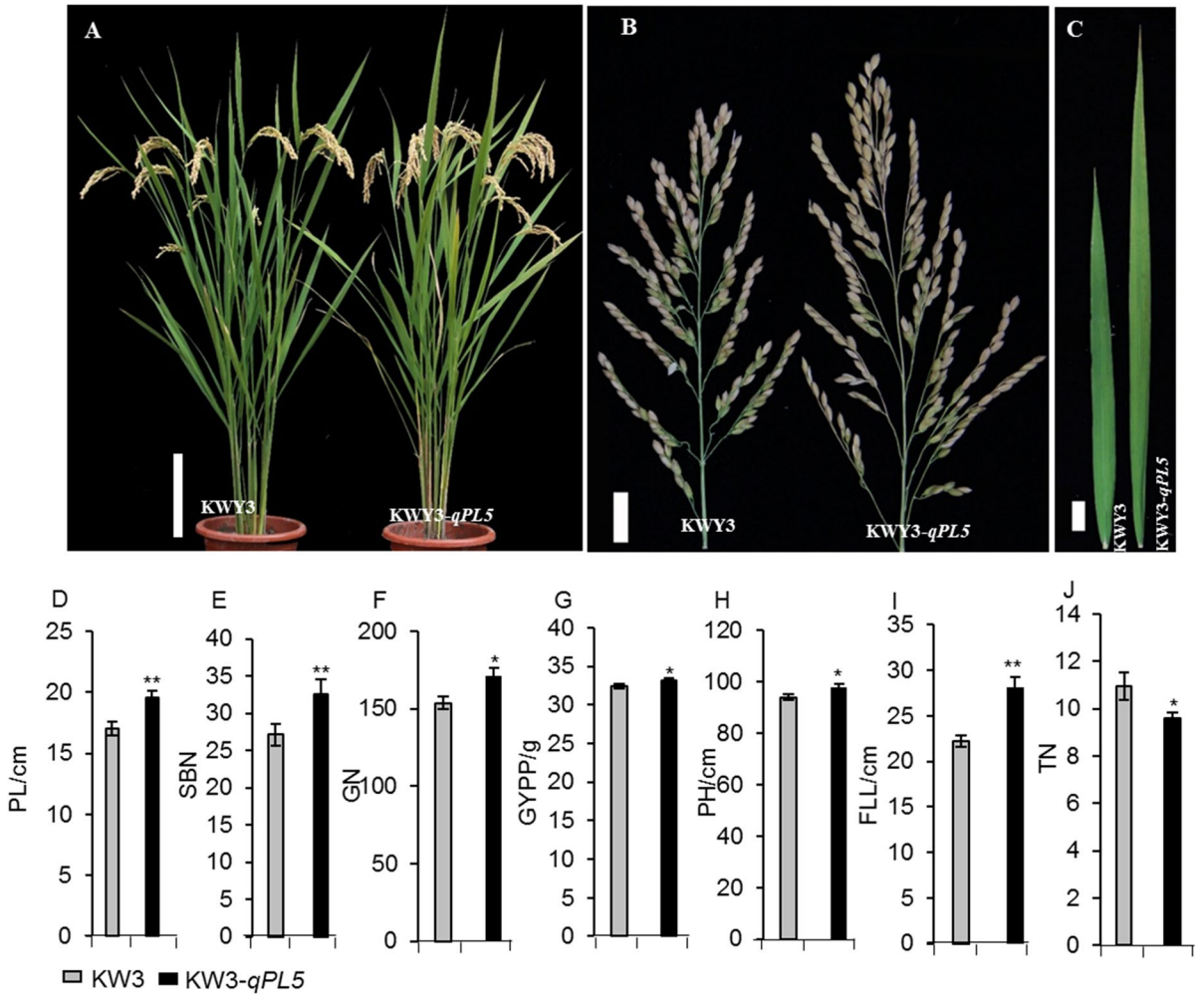


Fig. 5 Gross morphology of KWY3 (left) and KWY3-*qPL5* (right). **A** Mature plant phenotypes. **B**, **C** The appearance of panicles and flag leaves. **D**–**J** Comparisons of PL (panicle length), SBN (secondary branch number), GN (grain number per panicle), GYYP (grain yield per plant), PH (plant height), FLL (flag leaf length), and TN (tiller number) between KWY3 and KWY3-*qPL5*. Error bars represent the mean value ± SE of three biological replicates ($n = 50$ plants in **G**; $n = 6$ plants for the other traits). p values were calculated by one-way ANOVA. ** and * indicate significant differences at $p < 0.01$ and $p < 0.05$, respectively. Scale bars = 15 cm (**A**) and 2 cm (**B**, **C**)

Discussion

Increased productivity has long been one of the most important breeding targets in rice. To date, many QTLs for yield-related traits have been identified, and some of them have been used to develop varieties with high grain yield (Feng et al. 2017; Kim et al. 2018; Miura et al. 2011; Wang et al. 2020). Although PL has been reported to be positively associated with rice yield by regulating several panicle architecture related traits (Zhang et al. 2021), only a few PL-related genes/QTLs have been cloned or fine-mapped, and the molecular mechanisms underlying PL control are unknown. In our previous research, we identified *qPL5*, a PL QTL on Chr. 5, that leads to significantly increased PL in the NP genetic background (Xu et al. 2019). In this study, the location of *qPL5* was successfully narrowed down to a 21.86-kb region of chromosome 5, and LOC_Os05g41230 was predicated to be the most likely candidate gene for *qPL5*. We also evaluated the genetic effect of *qPL5* in different genetic backgrounds and showed that the

boost in grain yield that resulted from incorporating the '9311' allele of *qPL5* is due to drastic changes in panicle architecture and related traits.

PL is a typical complex quantitative trait that is controlled by multiple quantitative loci and is significantly influenced by environmental factors, which increases the difficulty of identifying PL QTL (Liu et al. 2011; Xing et al. 2002). Previous studies have shown that advanced genetic populations, NILs or CSSLs, show great potential in mapping QTLs, especially QTLs with minor genetic effects (Shibaya et al. 2016; Uga et al. 2007). In our previous study, we found *qPL5* that explained ~ 10% of the phenotypic variation for PL in the CSSL population, indicating that *qPL5* might be a minor-effect QTL (Xu et al. 2019). To avoid 'noise' due to the genetic background, N58, the CSSL carrying only one chromosomal segment introgressed from '9311', was selected as a parent to develop the segregating populations used in this study, and *qPL5* was found to explain 23.21% of the phenotypic variation in the N58/NP F_2 population. These results not only show that PL can be affected by genetic background, but also indicate that N58 is an ideal parent for fine mapping *qPL5*. Furthermore, to increase the accuracy of phenotypic characterization, we developed a set of introgression lines surrounding the *qPL5* locus and localized *qPL5* to an interval of 21.86 kb flanked by the molecular marker loci STS5-99 (~ 24.147 Mb) and STS5-106 (~ 24.168 Mb) on Chr. 5. At present, there are 16 reported PL QTLs on Chr. 5, but most have not been further characterized or fine mapped. Among these reported QTLs, one called *qPL5-1* has been detected in a region defined by molecular marker loci RM18626 and RM3089 in a CSSL population derived from the *indica* variety 'Guangluai 4' as recipient and NP as the donor, and this region overlaps the location of *qPL5*. Thus, further study will be required to determine whether *qPL5* and *qPL5-1* are the same QTL. Nonetheless, our estimate for the location of *qPL5* is more precise.

The analysis of ORFs in the mapping region predicted two putative ORFs, *LOC_Os05g41230* and *LOC_Os05g41240*, that encode BAK1 (Brassinosteroid insensitive 1-associated kinase 1) and a Myb-like DNA-binding domain-containing protein, respectively. In a previous study, the rice *BAK1* homolog, *OsI-BAK1*, was reported to interact with BRI1 for brassinosteroid perception and signal transduction to control the genetic regulation of PL, and the expression level of *Osl-BAK1* was positively correlated with PL (Khew et al. 2015). Sequence comparison showed that there are some variations in both the promoter region and the coding sequence of *LOC_Os05g41230*. In addition, the relative expression level of *LOC_Os05g41230* was higher in young panicles of NP-*qPL5* compared to NP. Thus, we speculate that *LOC_Os05g41230* is the most likely candidate gene for *qPL5*. Of course, the candidate gene must be subjected to verification by positional cloning and transformation.

Rice yield is a complex trait that is ultimately determined by three main factors: TGW, TN, and GN (Xing and Zhang 2010). Development of a NIL population is an appropriate strategy for confirming and evaluating the genetic effects of a QTL (Benson et al. 2015; Ding et al. 2011; Zhou et al. 2018). In the current study, we developed NILs in different genetic backgrounds, and the genetic backgrounds were quantified using the rice 40 k SNP array. Agronomic trait analysis of the NILs showed that *qPL5* did positively contribute to GN (grain number per panicle) but that its contribution to TN (tiller number) was negative. The GYPP (grain yield per plant) was significantly increased in the NILs, and this resulted from the increased GN. We noted that there were obvious differences in the relative increase in GYYP between NP/NP-*qPL5* (~ 15.13%) and KWY3/KW3-*qPL5* (~ 2.28%). This can be explained by the fact that KWY3 is an elite, high-yielding *japonica* variety with good-quality grain, and our result is consistent with results of previous studies showing that it is difficult to sharply increase grain yields in high yielding varieties (Zhang et al. 2009, 2014). In addition, *qPL5* can cause significant changes in PH (plant height) and FLL (flag leaf length), similar to the genetic effects of *qPL8* and *Ghd7.1* (Liu et al. 2013; Tang et al. 2018; Zhang et al. 2021). Furthermore, there were no significant differences in Hd (heading date) and grain-size-related traits between the NILs and their recipient parental lines, and this is different from the genetic effects of the cloned genes *PT2, OsGRF4*, and *DTH8*/*Ghd8* that control the regulation of PL (Sun et al. 2016; Xue et al. 2008; Yan et al. 2011). In breeding practice, PH is negatively correlated with lodging resistance, and we might need to pyramid *qPL5* with other genes controlling PH to outweigh the genetic effect of *qPL5* in future rice breeding (Wang

et al. 2016). Meanwhile, we will evaluate the quality traits of NILs, which would provide a comprehensive evaluation of the breeding value of *qPL5*. Nevertheless, we conclude that *qPL5* should be a valuable gene for breeding high-yield *japonica* varieties, and these results lay the foundation for gene cloning and breeding utilization of *qPL5* in the future.

Authors' contributions ZX and HZ analyzed the data and drafted the manuscript. ZX performed the phenotypic evaluation and data analysis with assistance from ML, YD, XL, and RW. ZC and XZ participated in the construction of the SLs. ST participated in the design of the study. QL and HZ designed the study and revised the manuscript. All of the authors have read and approved the final version of the manuscript.

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Data availability All data supporting the conclusions of this article are provided within the article (and its Additional files).

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors declare no conflicts of interest.

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