



Identification and fine mapping of *qGN1c*, a QTL for grain number per panicle, in rice (*Oryza sativa*)

Zuopeng Xu · Yixu Miao · Zhihai Chen · Hailin Gao · Ruixuan Wang · Dongsheng Zhao · Baochai Zhang · Yihua Zhou · Shuzhu Tang · Honggen Zhang · Qiaoquan Liu

Received: 25 April 2019 / Accepted: 14 August 2019 / Published online: 27 August 2019
© Springer Nature B.V. 2019

Abstract Grain number per panicle (GN) is one of the most important determinants of grain yield potential in rice. However, the underlying genetic and molecular mechanisms that regulate GN remain largely unknown. In this study, quantitative trait loci (QTLs) for grain yield-related traits were detected using a set of chromosomal segment substitution lines (CSSLs) that were generated from a cross between the *indica* cultivar 9311 as the trait donor, and the *japonica* cultivar ‘Nipponbare’ as the recipient. In total, 25 QTLs for

panicle-related traits, including GN, panicle length, primary branch number, and secondary branch number, were identified on eight chromosomes. Among the QTLs, *qGN1c* for GN was found to be located near *Gn1a*, a previously reported major QTL for GN on chromosome 1. Fine mapping placed *qGN1c* within a region of ~379 kb in a chromosomal interval flanking *Gn1a*, indicating that *qGN1c* is an uncloned gene. Evaluation of agronomic traits in a near isogenic line (NIL-*qGN1c*⁹³¹¹) suggested that *qGN1c* does not have additional impacts on agronomic traits except GN and thousand grain weight. More importantly, the grain yields per plant for the NIL-*qGN1c*⁹³¹¹ were significantly increased by 13.34% and 14.46% in two planting locations. Therefore, we conclude that the identification of *qGN1c* provides a useful genetic tool for the improvement of grain yield in rice breeding, and our findings will be helpful in cloning *qGN1c*.

Zuopeng Xu and Yixu Miao contributed equally to this work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11032-019-1039-7>) contains supplementary material, which is available to authorized users.

Z. Xu · Y. Miao · Z. Chen · H. Gao · R. Wang · D. Zhao · S. Tang · H. Zhang · Q. Liu
Jiangsu Key Laboratory of Crop Genetics and Physiology/Key Laboratory of Plant Functional Genomics of the Ministry of Education College of Agriculture, Yangzhou University, Yangzhou 225009, China

Z. Xu · Y. Miao · D. Zhao · S. Tang · H. Zhang (✉) · Q. Liu (✉)
Jiangsu Key Laboratory of Crop Genomics and Molecular Breeding/Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China
e-mail: zhg@yzu.edu.cn
e-mail: qqliu@yzu.edu.cn

B. Zhang · Y. Zhou
State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Keywords Rice · Grain yield · Grain number per panicle · *qGN1c* · QTL mapping

Introduction

Rice (*Oryza sativa* L.) is an important staple crop that feeds over one-half of the world's population. However, due to the ever-increasing population, food shortages have become a serious problem. Thus, it is essential to increase grain yield to meet the food demands required by a growing population (Godfray et al. 2010; Huang et al. 2013). Grain yield in rice is characterized by four

components, tiller number (TN), thousand grain weight (TGW), seed setting ratio (SSR), and grain number per panicle (GN). Of these, GN is traditionally considered to be one of the most important factors. At present, many studies have reported that some morphological component of panicle architecture such as panicle length (PL) and the number of primary and secondary branches (PBN/SBN) are important agronomic traits that affect GN (Luo et al. 2009; Mei et al. 2006; Wang et al. 2017). Hence, it is of great theoretical and practical value to identify key genes for GN and GN-related traits.

GN is a canonical complex trait controlled by a number of major and minor quantitative trait loci (QTLs), which presents a challenge in characterizing this trait (Xing et al. 2002). Although researchers have already extensively investigated the QTLs contributing to the regulation of GN in rice, favorable genes/QTLs that could be directly used in rice breeding remain rare. At present, at least 900 QTLs for GN have been identified on the 12 rice chromosomes (<http://www.gramene.org>), and some of them have been fine mapped. *GPA7*, a QTL for grain number, was delimited to a small region of ~35 kb located on chromosome 7 (Tian et al. 2006). *qGY2-1*, a QTL for gain yield, was fine mapped to an interval of ~102.9-kb on chromosome 2 (He et al. 2006). *SPP1*, a QTL that controls spikelet number, was fine mapped to a region of ~107-kb on the short arm of chromosome 1 (Liu et al. 2009). *qTSN12.2*, a QTL for total spikelet number per panicle, was fine mapped to an approximately 92 kb region on chromosome 12 (Sasaki et al. 2017). *GN4-1*, a major QTL responsible for GN on chromosome 4, was delimited to an interval of ~190-kb (Zhou et al. 2018). The QTL *qSPP2.2*, which controls spikelet number per panicle, was localized to an ~167.1-kb region on the long arm of chromosome 2 (Kaur et al. 2018). Several QTLs involved in the regulation of GN have already been cloned.

The first QTL isolated for GN was *Gn1a* that is located on the short arm of chromosome 1 and encodes a cytokinin oxidase/dehydrogenase (CKX) (Ashikari et al. 2005). CKX is the sole enzyme that catalyzes the irreversible degradation of cytokinins (CKs). Hence, decreased expression of *Gn1a* in inflorescence meristems can lead to the accumulation of CK, which finally results in increased GN (Hwang et al. 2012). A gain-of-function mutation in *DEP1* truncates a phosphatidylethanolamine-binding protein-like domain protein and increases the grain number, although the panicle length is reduced (Huang et al. 2009). The *IPA1/OsSPL14* gene encodes

squamosa promoter binding protein-like 14 and is a target of OsmiR156 (Jiao et al. 2010). The mutant allele that disrupts the OsmiR156-binding site causes increased GN. Further research has demonstrated that *IPA1* may be a positive regulator of *DEP1* (Lu et al. 2013). *NOG1* encodes an enoyl-CoA hydratase/isomerase and can increase grain yield by enhancing GN with no negative effects (Huo et al. 2017). *DTH7/Ghd7.1*, *Ghd7*, and *DTH8/Ghd8* are three major QTLs for heading date (HD) that can also lead to increased GN under long-day conditions (Gao et al. 2014; Liu et al. 2013; Wei et al. 2010; Weng et al. 2014; Yan et al. 2011). *GN2* exhibits pleiotropism and not only influences grain number but also affects plant height and heading date (Chen et al. 2017). *GNP1*, a gene that encodes gibberellin 20-oxidase, can enhance GN (Wu et al. 2016), suggesting that GA is an important hormone in the regulation of GN.

As mentioned above, although some QTLs for GN-related traits have been mapped or cloned, the underlying genetic and molecular mechanisms that regulate GN are still unknown. It is therefore worthwhile to exploit new genetic loci that could increase GN. In this study, we first identified QTLs responsible for GN regulation using a set of chromosomal segment substitution lines (CSSLs) derived from a cross between the *japonica* cultivar ‘Nipponbare’ (‘NP’) and the *indica* cultivar 9311. Among the identified QTLs, a major QTL, *qGN1c*, located on chromosome 1 (Chr. 1) was fine mapped to a chromosomal interval of ~379 kb delimited by flanking marker loci STSN2-17 and STSN2-18 using advanced BC_nF₂₋₅ segregating populations. The genetic effect of *qGN1c* was also evaluated using near-isogenic lines (NILs). The results of our study provide an opportunity to clone *qGN1c*, which will be useful for improving grain yield in rice breeding.

Materials and methods

Plant materials and population development

A set of 125 CSSLs that together cover 95.6% of the 9311 genome were generated by using 9311, an *indica* cultivar, as the segment donor, and ‘Nipponbare’ (‘NP’), a *japonica* cultivar, as the recipient. Each of the CSSLs carries one or a few donor chromosomal segments (Xu et al. 2017; Zhang et al. 2011). In the summer of 2014 and 2015, the CSSLs and their parental lines were grown and phenotyped to identify QTLs for panicle-

related traits. One CSSL line, N2, was selected to cross with ‘NP’ to develop the segregating population for *qGNIc* validation. In the summer of 2016, an F₂ population containing 300 plants was developed. Of the F₂ plants, 282 individuals were selected to genotype and phenotype for genetic analysis. In the winter of 2016, the recombinant individuals (F₃ plants) were grown and genotyped to identify individual plants with homozygous genotypes (9311 type). In the summer of 2017, six types of recombinant individuals (F₄ generation) with homozygous genotypes (9311 type) were planted and phenotyped for the initial mapping of *qGNIc*. In addition, 25 individual plants that were found to be heterozygous in the mapped region were selected from the F₂ population to produce a large segregating population for the fine mapping of *qGNIc*. In the summer of 2017, 2820 plants were chosen for the detection of recombination events using the molecular markers STSN2-17 and STSN2-20. In the winter of 2017, six types of recombinant individuals (F₄ generation) were planted and genotyped. In the summer of 2018, six types of recombinant individuals (F₅ generation) with homozygous genotypes (9311 type) were planted and phenotyped for fine mapping *qGNIc*. A set of near-isogenic lines (NILs) carrying *qGNIc* in the ‘NP’ background (NIL-*qGNIc*⁹³¹¹) were also developed using a backcross scheme with marker-aided selection. In the summer of 2018, 200 individual plants from the NIL-*qGNIc*⁹³¹¹ population and NP were selected to evaluate the effect of *qGNIc* on yield in two planting locations.

Field experiments

The rice lines were planted in the experimental fields at Xiao Nei (XN; 32° 23′ 24″ N, 119° 25′ 3″ E) and Jiu Dian (JD; 32° 29′ 54″ N, 119° 26′ 4″ E), Yangzhou, Jiangsu Province, China, in the summer. In the winter, the lines were grown in the fields at Lingshui (18° 31′ 52″ N, 110° 10′ 53″ E), Hainan Province, China. The growing density was 15 × 25 cm for line × row.

Measurement of grain yield-related traits and data analysis

We evaluated a total of 14 agronomic traits, including plant height (PH), heading date (HD), tiller number per plant (TN), seed setting ratio (SSR), grain number per panicle (GN), panicle length (PL), primary branch

number (PBN), secondary branch number (SBN), primary branch grain number (PBGN), secondary branch grain number (SBGN), thousand grain weight (TGW), grain length (GL), grain width (GW), and grain yield per panicle (GYPP). These agronomic traits were analyzed using a previously described method (Liu et al. 2016b; Wang et al. 2017; Xu et al. 2017; Yu et al. 2017). In brief, panicles in the tallest tillers were harvested and measured to evaluate the panicle-related trait phenotypes. We chose 10–15 individuals from the middle of the row in each line for the evaluation of all phenotypes except for GYPP. GYPP was measured as the mean weight of grain from 200 individual plants. SPSS software (version 15.0) was used to analyze the correlation coefficients among grain yield-related traits, and the other data for each trait were analyzed using an independent sample *t* test program.

DNA extraction and molecular analysis

Genomic DNA was extracted from fresh rice leaves of one plant using the CTAB method (Murray and Thompson 1980). PCR was performed and PCR products separated using a previously described method (Wu et al. 2009). The novel InDel molecular markers that were used for validation and fine mapping of *qGNIc* in this study were developed using PRIMER 5.0 software based on the publicly available rice genome sequence (<http://www.ncbi.nlm.nih.gov/>). DNA sequences of primers used to amplify the novel InDel markers are given in Supplemental Table 1; Gnl1a-M is a gene-tagged marker based on a 16-bp deletion in the 5′-untranslated region of *Gnl1a* in the cultivars ‘Habataki’ and 5150 (Yan et al. 2009).

QTL analysis

The QTLs identified in this study were mapped using the software ICI-Mapping 4.0 with the aid of the Binmap, in which the 9311 and ‘NP’ genotypes were assigned the values 2 and 0, respectively (Meng et al. 2015; Xu et al. 2017). QTL analysis was performed using the inclusive composite interval mapping (ICIM) method, and the threshold of the logarithm of the odds ratio (LOD) was 2.5. The QTLs were named using the nomenclature as given in McCouch and CGSNL (2008).

Results

Variation in panicle characteristics between the parental lines and the CSSL population

GN is one of the most prominent differences between ‘NP’ and 9311. GN in 9311 is approximately double that in ‘NP’ (Fig. 1a, b). GN is affected by panicle length (PL), primary branch number (PBN), and secondary branch number (SBN) (Luo et al. 2009; Mei et al. 2006; Wang et al. 2017). Therefore, to address this difference in GN, PL, PBN, and SBN were further investigated in the parental lines. The values for PL, PBN, and SBN were also significantly higher in 9311 compared with ‘NP’ (Fig. 1c–e).

To understand the molecular basis of the regulation of GN, we investigated the behaviors of the three factors in the CSSL population and performed QTL analyses. A continuous distribution frequency of these factors confirmed that GN is a complicated trait controlled by multiple QTLs (Fig. 2). Except for the distribution frequency of PBN (Fig. 2c), the values for GN, PL, and SBN were comparable in 2 years, implying that PBN is more sensitive to environmental effects. Although these GN-related factors in the majority of the lines were similar to those in ‘NP’, several lines exhibited improved phenotypes, indicating that some of the introgressed segments harbor elite loci. Correlation analysis showed that the measured traits in this study were significantly positively correlated with each other except for PBN and SBN (Table 1). A striking correlation (correlation coefficient > 0.88) was found between SBN and GN, indicating that SBN is tightly associated with GN.

QTL mapping

The ICIM approach was used to detect QTLs for panicle-related traits in the CSSL population based on 2 years of phenotypic investigations. Twenty-five QTLs were found to be distributed on eight chromosomes; chromosomes 4, 10, 11, and 12 did not have any QTLs for GN (Table 2; Supplemental Fig. 1). In 2014, a total of 12 QTLs, six for PL, three for SBN, and three for GN, were found on six chromosomes and explained 64.45%, 32.60%, and 42.21% of the phenotypic variance, respectively. Among these QTLs, *qPL5*, *qPL6-3*, *qPL9-1*, *qSBN6*, *qGN1c*, and *qGN6* were considered to be major QTLs because they could explain > 10% of the

phenotypic variance. Interestingly, three QTL clusters located on Chr. 1, 6, and 9 were simultaneously identified to control other panicle-related traits (Table 2; Supplemental Fig. 1), which is in agreement with the correlation analysis (Table 1). In fact, the regions containing the clustered QTLs mapped on Chr. 6 and 9 include two previously reported QTLs for PL, *qPL6* and *Lp1*, respectively (Liu et al. 2016a; Sun et al. 2017) and the region containing *qPL7-1* contains the *Ghd7* gene (Xue et al. 2008). The mapped region on Chr. 1 containing another QTL cluster is near *Gn1a* but does not include *Gn1a* (Ashikari et al. 2005), because three gaps were found on Chr. 1 and the *Gn1a* locus is just inside gap 1 (Supplemental Fig. 1; Supplemental Fig. 2). Therefore, we speculate that this mapped region may harbor novel genes for determining GN. Of the 13 QTLs identified in 2015, six for PL, one for PBN, three for SBN, and three for GN were found to explain 53.86%, 8.47%, 38.29%, and 41.84% of the phenotypic variance, respectively (Table 2). In particular, we also identified four major QTLs, including *qPL6-4*, *qSBN6*, *qGN1c*, and *qGN6*, as well as three QTL clusters located on Chr. 1, 2, and 6 (Supplemental Fig. 1). Notably, the mapped QTL cluster located on Chr. 2 is near *DTY2.1*, a previously-reported locus for grain yield (Venuprasad et al. 2009).

Taken together, the majority (18/25) of the identified QTLs had positive additive effects, implying that these alleles from 9311 may be directly used in the design of molecular breeding strategies for high grain yield (Table 2). Moreover, based on the mapped region, five QTLs, *qPL5*, *qSBN1*, *qSBN6*, *qGN1c*, and *qGN6*, were found to be co-localized. In addition, two QTL pairs (*qPL6-1* and *qPL6-2* as well as *qPL6-3* and *qPL6-4*) for PL were also identified as being in close proximity to each other with similar additive effects (Table 2; Supplemental Fig. 1). We therefore considered that these QTLs were worthy of further analysis. Only one QTL for PBN was detected in 2 years, suggesting that this trait is sensitive to environmental effects.

Validation and fine mapping of *qGN1c*

Given that *qGN1c* is a new locus that has a positive contribution to GN, we next performed fine mapping to validate this QTL. Re-sequencing data of the CSSL population showed that CSSL line N2 carrying *qGN1c* had a single 9311-derived fragment introgression (2,141,284 to 5,084,973 bp) on Chr. 1 (Fig. 3a). Seven

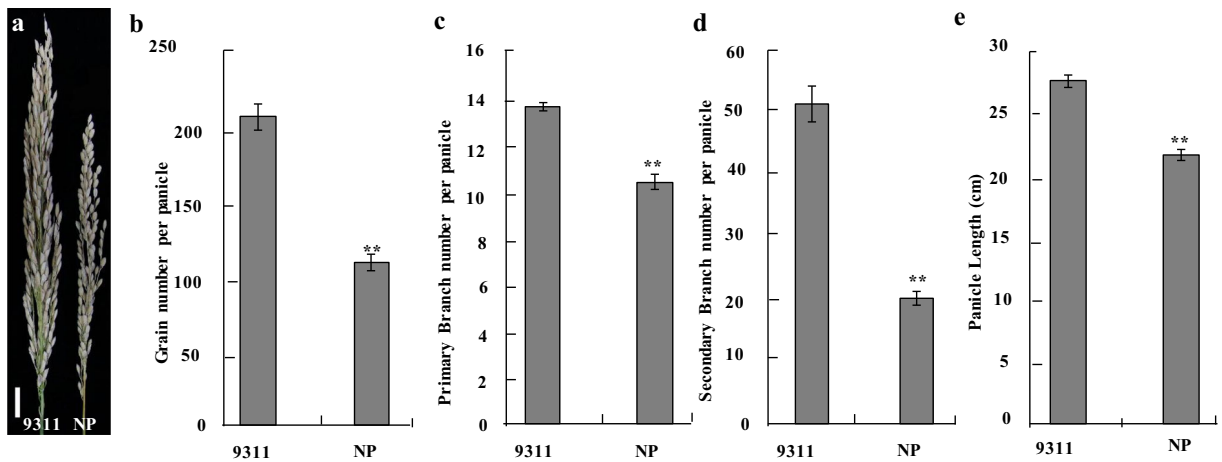


Fig. 1 Phenotypic differences in panicles of 9311 and ‘NP’ (‘Nipponbare’). **a** Panicle morphology, scale bar=2 cm. **b–e** Comparisons of four panicle-related traits between 9311 and

‘NP’. Error bars represent the mean value \pm SE ($n = 10$ plants). ** and * indicate significant differences at the $P < 0.01$ and $P < 0.05$ levels, respectively, as determined by the two-tailed t test

polymorphic marker loci distributed in the region from 2.1 to 5.2 Mb were developed, and the physical location of the 9311 chromosomal segment was confirmed in the N2 line (Supplemental Table.1; Supplemental Fig. 3). N2 plants had higher GN than did ‘NP’ plants (Fig. 3c, d). We next investigated five GN-related traits,

including PL, PBN, SBN, PBGN (primary branch grain number), and SBGN (secondary branch grain number), in ‘NP’ and N2. SBN and SBGN were significantly increased in N2 (Fig. 3f, g), resulting in higher GN in N2 than in ‘NP’, whereas PL, PBN, and PBGN, as well as plant height (PH) and heading date (HD), were

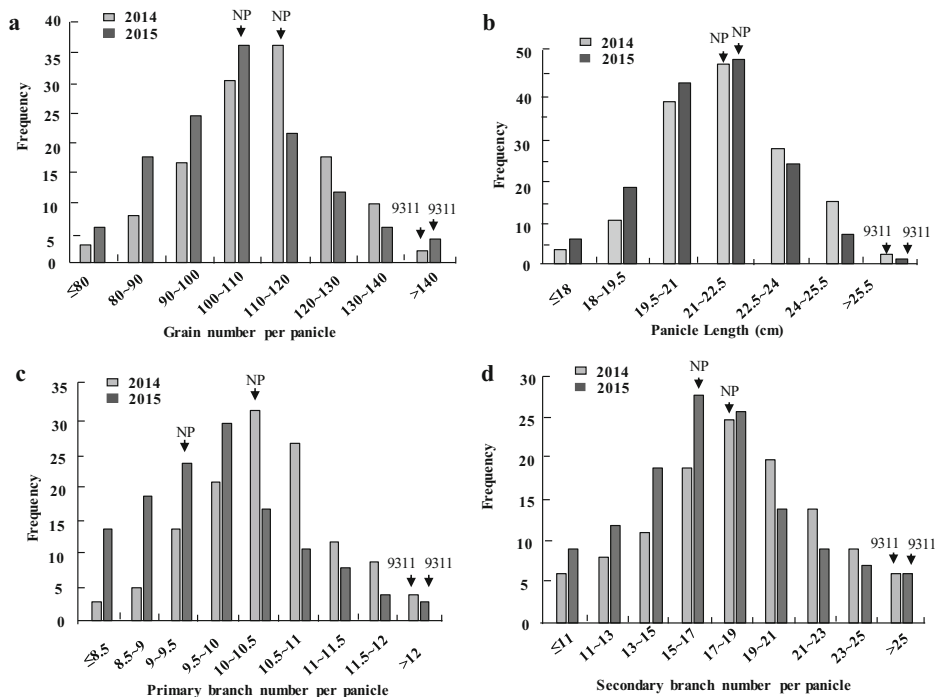


Fig. 2 Frequency distribution of panicle-related traits in the CSSLs and the parental inbred lines (‘NP’ and 9311) in field seasons 2014 and 2015. **a** Grain number per panicle, **b** panicle

length, **c** primary branch number per panicle, and **d** secondary branch number per panicle. The values for the two parental lines (‘NP’ and 9311) are indicated by black arrows in each panel

Table 1 Correlation coefficients among four panicle-related traits

Traits	PL	PBN	SBN	GN
PL	1			
PBN	0.32**	1		
SBN	0.43**	0.081	1	
GN	0.48**	0.26**	0.88**	1

PL, panicle length; PBN, primary branch number; SBN, secondary branch number; GN, Grain number per panicle. ** and * indicate significant differences at the $P < 0.01$ and $P < 0.05$ levels, respectively, as determined by the two-tailed t test

identical in N2 and ‘NP’ (Fig. 3b, c, e–i). Taking all of these findings together, we chose N2 as a parent in a cross with ‘NP’ to generate a fine-mapping population.

After phenotyping and genotyping the 282 individual plants of an F_2 population derived from N2 and ‘NP’ (Supplemental Table 1; Fig. 4A), we found that the plants with 9311 homozygous genotype (group III) yielded more grain numbers, and the heterozygous

genotype (group II) had grain numbers between ‘NP’ (group I) and the plants that were homozygous for $qGN1c$ (Fig. 4b, c). These results showed that $qGN1c$ is a real QTL for GN and that it is semi-dominant. We also identified six types of recombinants in the F_2 families based on the positions of recombination breakpoints determined using molecular markers, and individual recombinant F_4 plants with homozygous 9311 genotypes were selected for quantification of the GN phenotype (Fig. 4d). Compared with ‘NP’, plants in groups III, IV, VI, and VII had significantly increased GN. In contrast, no significant differences were observed between ‘NP’ and plants in groups II and V (Fig. 4c). Therefore, combining the genotypes and phenotypes of the six groups allowed us to map $qGN1c$ to a ~ 1.14-Mb genomic region flanked by marker loci STSN2-17 and STSN2-20 (Fig. 4d).

To further narrow down the mapped region, 25 F_2 heterozygous plants were used to develop a self-population for fine mapping. By screening 2820 F_3

Table 2 Identification of QTLs for four panicle-related traits by composite interval mapping in 2014 and 2015

Trait	QTL	Bin	Position (Mb)	CSSL line	2014			2015		
					LOD	PVE (%)	Add	LOD	PVE (%)	Add
PL	$qPL1$	Bin15	37.95–40.08	N9	–	–	–	3.27	6.97	–2.75
	$qPL5$	Bin122	21.49–24.21	N22,N58	4.91	11.97	1.74	2.91	7.61	1.36
	$qPL6-1$	Bin129	2.81–3.77	N6,N60–N64,N67	4.88	9.36	–1.03	–	–	–
	$qPL6-2$	Bin130	3.77–3.80	N6, N60–N65, N107	–	–	–	3.99	8.62	–1.05
	$qPL6-3$	Bin151	21.01–21.3	N66–N68,N130	5.00	13.47	1.33	–	–	–
	$qPL6-4$	Bin152	21.3–21.55	N66,N68–N69,N130	–	–	–	6.93	15.98	1.89
	$qPL6-5$	Bin158	25.89–26.83	N66, N70	4.71	8.97	1.87	–	–	–
	$qPL7-1$	Bin188	7.81–9.94	N79, N80	3.81	7.89	1.95	–	–	–
	$qPL7-2$	Bin196	19.40–20.58	N77, N79, N81–N84	–	–	–	4.01	8.67	1.05
	$qPL9-1$	Bin268	18.33–18.74	N8, N107–N109	4.83	12.79	–1.58	–	–	–
$qPL9-2$	Bin273	21.62–23.63	N8, N108	–	–	–	2.84	6.01	–1.81	
PBN	$qPBN8$	Bin221	4.57–5.36	N23, N91–N94	–	–	–	4.26	8.47	1.03
SBN	$qSBN1$	Bin6	2.60–4.80	N1, N2, N3, N10	3.80	9.68	3.92	3.32	9.31	3.51
	$qSBN2$	Bin25	6.63–9.86	N13,N14,N132,N133	–	–	–	2.88	8.47	3.52
	$qSBN3$	Bin56	3.36–3.82	N26,N28,N30, N129	3.30	8.99	–2.99	–	–	–
	$qSBN6$	Bin152	21.3–21.55	N66,N68–N69,N130	5.37	13.92	3.66	6.74	20.51	4.89
GN	$qGN1c$	Bin6	2.60–4.80	N1, N2, N3, N10	3.52	15.73	2.21	2.80	10.31	9.64
	$qGN2$	Bin25	6.63–9.86	N13,N14,N132,N133	–	–	–	2.57	8.11	2.98
	$qGN6$	Bin152	21.3–21.55	N66,N68–N69,N130	5.81	17.61	3.75	7.21	23.42	21.65
	$qGN9$	Bin268	18.33–18.74	N8, N107–N109	3.11	8.87	–1.67	–	–	–

Bin, bin overlapping with the LOD peak of the QTL; LOD, logarithm of the odds ratio; PVE, percentage of the trait variance explained by the QTL; Add, the allelic effect is calculated as the mean effect of replacing ‘NP’ alleles by 9311 alleles at the QTL

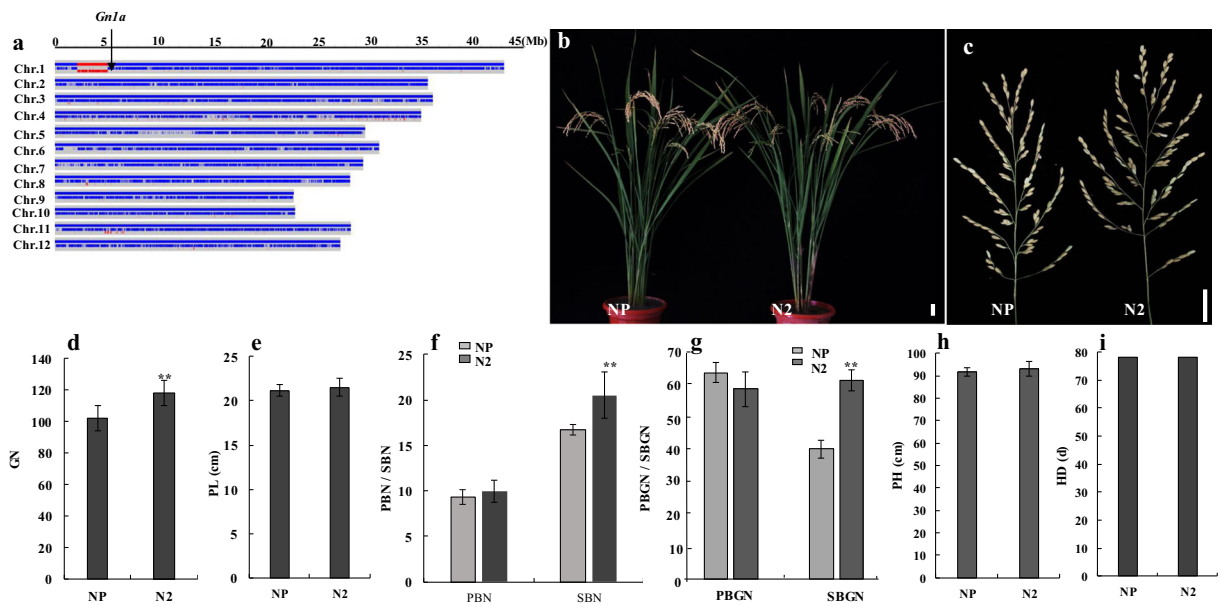


Fig. 3 Phenotypic differences between ‘NP’ and the CSSL line N2 in 2014. **a** Graphical genotype of CSSL line N2. The position of the introgressed chromosomal segment from 9311 is shown in red. The location of *Gnl1a* is indicated by an arrow. **b** Mature plants of ‘NP’ and N2. **c** Panicle phenotypes. **d–i** Comparisons of GN, PL, PBN, SBN, PBGN (primary branch grain number), SBGN

(secondary branch grain number), PH (plant height), and HD (heading date) between ‘NP’ and N2. Error bars represent the mean value \pm SE ($n=10$ plants). ** and * indicate significant differences at the $P < 0.01$ and $P < 0.05$ levels, respectively, as determined by the two-tailed t test. Scale bar = 5 cm in **b** and 2 cm in **c**

generation individuals, we identified 42 recombinants based on the STSN2-17 and STSN2-20 marker alleles (Fig. 4e). Using four additional polymorphic markers, the 42 recombinants were further classified into six groups based on the positions of the recombination breakpoints (Fig. 4E). The results of a progeny test (F_5 generation) of these recombinants revealed that the plants in groups II–V had increased GN, while plants in groups VI and VII had reduced GN (Fig. 4C). Ultimately, the position of *qGN1c* was narrowed to within a ~ 379 -kb region of Chr. 1 flanked by marker loci STSN2-17 and STSN2-18 (Fig. 4e).

Performance of NIL-*qGN1c*⁹³¹¹

To evaluate the genetic effects of *qGN1c*, we identified a NIL for *qGN1c* (NIL-*qGN1c*⁹³¹¹) carrying a ~ 1.51 -Mb chromosomal segment introgressed from 9311 flanked by marker loci STSN2-15 and STSN2-20 (Fig. 5a). No significant differences were observed for PL, PBN, PBGN, HD, PH, SSR, TN, and grain width (GW) between NIL-*qGN1c*⁹³¹¹ and ‘NP’ plants. Similar to the CSSL line N2, NIL-*qGN1c*⁹³¹¹ plants produced higher SBN and SBGN (Fig. 5c; Table 3), resulting in GN increases of 15.92% and 18.61% in the Xiao Nei

(XN) and Jiu Dian (JD) environments, respectively. Compared with ‘NP’, grain yield per plant (GYPP) in NIL-*qGN1c*⁹³¹¹ was increased by 13.34% and 14.46% in the two locations (Table 3). Due to a reduction in grain length (GL), the TGW of NIL-*qGN1c*⁹³¹¹ was significantly decreased by an average of 6.43% and 5.51% compared with ‘NP’ (Fig. 5d; Table 3), but this did not affect GYPP. These results indicate that *qGN1c* from 9311 can enhance grain yield with no significant negative effects on other agronomic traits. Therefore, *qGN1c* may be a favorable allele for breeding high-yield rice cultivars.

Discussion

Grain yield is a complex trait that is controlled by a combination of GN, TGW, SSR, and TN; GN is typically the largest and most important factor for improvement of grain yield in rice breeding. Similarly, GN is also a complex trait that is likely to be affected by PBN, SBN, and PL. In addition to the influences of genetic variations, environmental conditions may also have a significant effect on this trait, which makes it challenging to characterize GN (Xing et al. 2002). Consistent

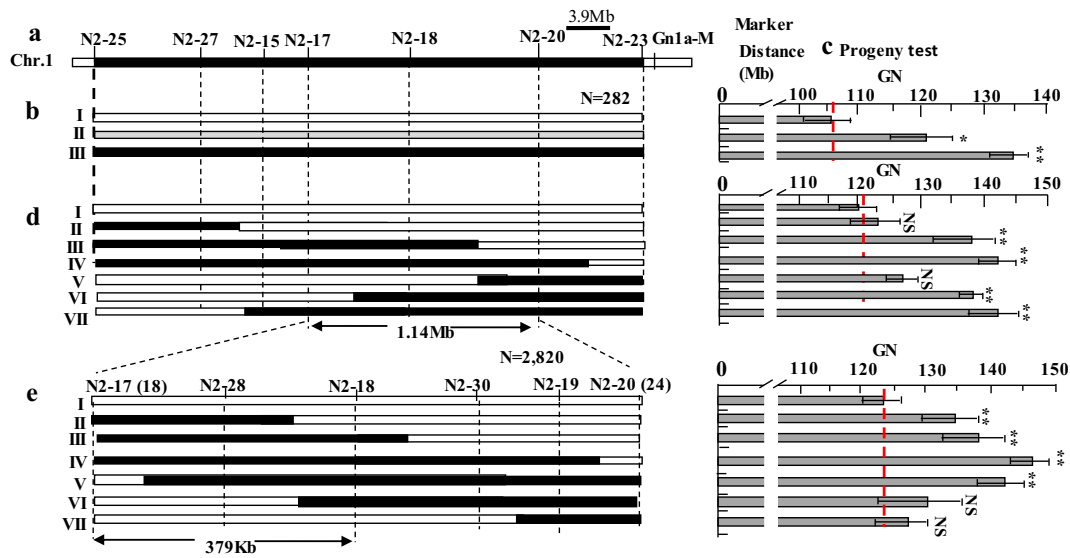


Fig. 4 Fine-mapping of *qGN1c*. **a** The physical location of the 9311 donor chromosomal segment on Chr. 1 in CSSL line N2. N2-15 to N2-25 indicate the locations of sequence-tagged site (STS) molecular marker loci. Gn1a-M is a gene-tagged marker specific for *Gn1a*. The genomic region from 9311 is shown as a black rectangle, and the genome of ‘Nipponbare’ (NP) is shown as white rectangles. **b** and **c** Genetic analysis using the subpopulation derived from the cross of ‘NP’ and N2. Representative genotypes of the segregating lines are shown on the left (**b**) and the GN values for the corresponding plants are shown on the right (**c**). The black, white, and gray rectangles indicate the homozygous 9311 genotype, the homozygous ‘NP’ genotype, and the heterozygous

genotype, respectively. **d** and **e** Primary mapping of *qGN1c* based on the genotypes (left) and phenotypes (right) of recombinants in an F₄ population. **e** Fine mapping of *qGN1c* using the F₅ population. The numbers in parentheses next to the marker loci represent the number of recombinant individuals, and the ~379-kb region containing *qGN1c* is indicated below the figure in **e**. The homozygous 9311 and homozygous ‘NP’ genotypes are shown as black and white rectangles, respectively. Error bars represent the mean value ± SE (*n* = 30 individuals in the F₂ population, 15 individuals in the substitution line). ** and * indicate significant differences at the *P* < 0.01 and *P* < 0.05 levels, respectively, as determined by the two-tailed *t* test

with the complexity of GN, a large number of genes/QTLs have been inferred to be involved in the regulation and control of GN. To date, several different mapping populations consisting of F₂, recombinant inbred lines (RILs), backcross inbred lines (BILs), and CSSLs, have been used to detect QTLs for complex traits, and

CSSL populations have been shown to be ideal permanent genetic resources for identification, fine mapping, and cloning of QTLs underlying complex traits (Ali et al. 2010; Qi et al. 2018; Tang et al. 2018; Xing et al. 2008; Zhou et al. 2009). It is widely accepted that mapping populations developed from parental lines with

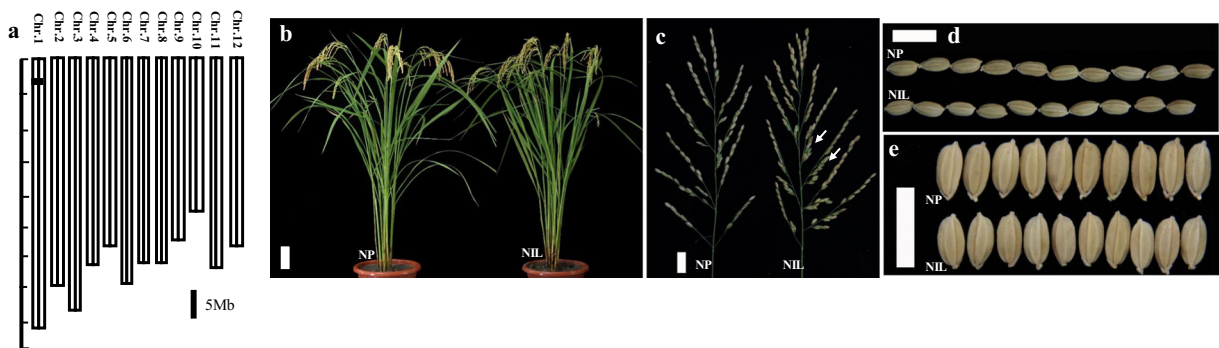


Fig. 5 The genotype and phenotype of NIL-*qGN1c*⁹³¹¹. **a** Graphical karyotype showing the physical location of the 9311 donor chromosomal segment on Chr. 1 in NIL-*qGN1c*⁹³¹¹. Comparisons

of gross morphology of mature plants (**b**) and panicles (**c**), grain length (**d**), and grain width (**e**) in ‘NP’ and NIL-*qGN1c*⁹³¹¹. Scale bars = 10 cm in **b**, 2 cm in **c**, and 1 cm in **d** and **e**

Table 3 Agronomic traits for ‘NP’ and NIL-*qGN1c*⁹³¹¹ plants grown in two locations in 2018

Environment	Line	GN	PL (cm)	PBN	SBN	PBGN	SBGN	SSR (%)
XiaoNei	NP	124.40 ± 18.86	22.40 ± 0.86	10.60 ± 1.67	22.81 ± 4.08	59.45 ± 8.17	64.95 ± 8.06	87.24 ± 2.35
	NIL- <i>qGN1c</i> ⁹³¹¹	144.20 ± 13.87**	21.76 ± 0.93	11.20 ± 0.83	28.97 ± 2.65**	63.42 ± 9.16	80.78 ± 7.65**	85.33 ± 3.32
JiuDian	NP	108.72 ± 20.17	21.89 ± 0.54	10.76 ± 1.23	21.76 ± 5.89	52.65 ± 6.15	56.07 ± 5.12	85.16 ± 2.95
	NIL- <i>qGN1c</i> ⁹³¹¹	128.95 ± 17.98**	21.34 ± 0.87	10.79 ± 1.15	27.85 ± 4.32**	59.65 ± 5.12	69.35 ± 8.16**	84.98 ± 1.89

Environment	TGW (g)	GL (mm)	GW(mm)	GYPP (g)	PH (cm)	HD (day)	TN
XiaoNei	23.78 ± 0.93	7.21 ± 0.64	3.00 ± 0.31	21.51 ± 4.52	96.17 ± 3.42	83.25 ± 0.12	13.81 ± 4.26
	22.25 ± 0.59*	6.86 ± 0.79*	3.02 ± 0.51	24.38 ± 5.91*	95.42 ± 2.17	82.50 ± 0.08	12.72 ± 3.15
JiuDian	23.05 ± 0.87	7.22 ± 0.98	3.10 ± 0.30	20.89 ± 3.78	93.12 ± 5.13	82.50 ± 0.03	11.56 ± 2.78
	21.78 ± 0.78*	6.91 ± 0.76*	3.05 ± 0.48	23.91 ± 4.32*	92.65 ± 7.27	82.00 ± 0.00	11.78 ± 3.65

The data for GN, PL, PBN, SBN, PBGN, SBGN, SSR (seed setting rate), PH, HD, and TN (tiller number) are given as mean ± SD ($n = 10$). The data for TGW (thousand grain weight), GL (grain length), and GW (grain width) are given as mean ± SD ($n = 100$ grains harvested from at least 10 individual plants). The data for GYPP (grain yield per panicle) is given as mean ± SD ($n = 200$). ** and * indicate significant differences at the $P < 0.01$ and $P < 0.05$ levels, respectively, as determined by the two-tailed t test

significant differences in the targeted traits are more effective for QTL identification (Ashikari et al. 2005). *Oryza sativa indica* and *Oryza sativa japonica* are two subspecies of rice. Genetic variations between cultivars of the two subspecies have been widely used in rice breeding (Qi et al. 2018). ‘NP’ and 9311 are representative *japonica* and *indica* rice cultivars, respectively, which display characteristics typical of the two subspecies. Hence, the CSSL population produced by introgressing 9311 genomic fragments into ‘NP’ was ideal for identifying and characterizing QTLs for GN and GN-related traits in our study. In fact, this population was used previously to dissect other grain yield related traits, such as TGW, GL, and GW (Xu et al. 2017).

In our study, we used the CSSL population to dissect the QTLs for panicle-related traits. A total of 25 QTLs for panicle-related traits were identified based on data collected over 2 years. A comparison of the location of QTL regions identified in the 2 years should clearly show which QTLs could be stably inherited. *qPL5*, *qSBN1*, *qSBN6*, *qGN1c*, and *qGN6* were found to be co-localized; *qPL6-1* and *qPL6-2* or *qPL6-3* and *qPL6-4* were close to each other with similar additive effects, indicating that these QTLs are stably inherited loci. *qSBN1* and *qGN1c*, as well as *qSBN6*, *qGN6*, and *qPL6-4*, were found to be located in the same regions, which is consistent with the correlation analysis and indicates that the corresponding gene may regulate several panicle-related traits. Three QTLs (*qSBN6*, *qGN6*, and *qPL6-4*) co-localize with the previously reported QTL *qPL6* (Sun et al. 2017), suggesting that the QTLs identified in this study are reliable. Moreover, several new QTLs, such as *qPL5*, *qPL6-1*, and *qPL6-2*, were identified in this study, and these will further our understanding of the mechanisms that control GN in rice.

qGN1c identified here is a major QTL for GN, and it mapped to the distal region of the genome containing the cloned gene *Gn1a* (Ashikari et al. 2005). Genotyping using the gene-tagged marker (Gn1a-M) for *Gn1a* revealed that the CSSL line N2 carrying *qGN1c* does not contain the 9311-type *Gn1a*. By using the population generated from N2 and ‘NP’, the *qGN1c* for GN was fine mapped to an interval of ~379-kb on Chr. 1, which is in the upper region of *Gn1a*. To date, several QTLs located near *Gn1a* have been reported, such as *SPP1* and *Gn1b*. *SPP1* has been fine mapped to the down region of *Gn1a* indicating that *qGN1c* and *SPP1* are two different genes (Liu et al. 2009). *Gn1b* is

located in the upper region of *Gn1a*, but the actual physical location of *Gn1b* is ambiguous (Ashikari et al. 2005). Thus, further study is needed to determine whether or not *qGN1c* and *Gn1b* are identical genes.

Currently, although a number of QTLs/genes for GN have been mapped and/or cloned, many of them show pleiotropic effects on other important agronomic traits. For example, The QTL *qGN4.1* for GN has pleiotropic effects on agronomic traits such as the number of tillers per plant and flag leaf length and width (Deshmukh et al. 2010). The QTL *qSPP2.2*, derived from *O. longistaminata*, showed a positive additive effect on GN, but decreased the number of tillers per plant (Kaur et al. 2018). The QTL *TSN12.2* for total spikelet number affects the morphology of both leaves and internodes (Sasaki et al. 2017). *qSPP7/DTH7/Ghd7.1*, *Ghd7*, and *DTH8/Ghd8*, three major cloned QTLs for grain yield, have also been reported to affect heading date and leaf size (Gao et al. 2014; Liu et al. 2013; Tang et al. 2018; Wei et al. 2010; Weng et al. 2014; Xing et al. 2008; Yan et al. 2011). *IPA1*, *DEP1*, and the fine-mapped QTL *qGN4* for grain number simultaneously regulate panicle size (Huang et al. 2009; Jiao et al. 2010; Zhou et al. 2018). The genome of NIL-*qGN1c*⁹³¹¹ harbors a ~1.51-Mb chromosomal fragment that was introgressed from 9311 into the ‘NP’ background, and this NIL shows increased GN without significant negative effects on PH, HD, SSR, and TN. The GYPP of NIL-*qGN1c*⁹³¹¹ was thereby increased by ~13% compared with ‘NP’, suggesting the potential of this locus for grain yield improvement in rice breeding. Taken together, these findings extend our understanding of the factors that control GN and also provide a tool to improve grain yield in rice production.

Author contributions Q.L., S.T., and Y.Z. conceived the study; Z.X., M.Y., Z.C., H.G., R.W., D.Z., and H.Z. performed the experiments and analyzed the data; Q.L., H.Z., and B.Z. reviewed and edited the article; and Z.X. wrote the article.

Funding information This study was supported by the National Natural Science Foundation of China (Grant Nos. 31771743 and 31701393), the Postdoctoral Science Foundation of China (Grant No. 2018M632395), and the Government of Jiangsu Province (BE2018357 and PAPD).

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

References

- Ali ML, Sanchez PL, Yu SB, Lorieux M, Eizenga GC (2010) Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa*). *Rice* 3:218–234
- Ashikari M, Sakakibara H, Lin SY, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. *Science* 309:741–745
- Chen H, Tang YY, Liu JF, Tan LB, Jiang JH, Wang MM, Zhu ZF, Sun XY, Sun CQ (2017) Emergence of a novel chimeric gene underlying grain number in rice. *Genetics* 205:993–1002
- Deshmukh R, Singh A, Jain N, Anand S, Gacche R, Singh A, Gaikwad K, Sharma T, Mohapatra T, Singh N (2010) Identification of candidate genes for grain number in rice (*Oryza sativa* L.). *Funct Integr Genomic* 10:339–347
- Gao H, Jin MN, Zheng XM, Chen J, Yuan DY, Xin YY, Wang MQ, Huang DY, Zhang Z, Zhou KN, Sheng PK, Ma J, Ma WW, Deng HF, Jiang L, Liu SJ, Wang HY, Wu CY, Yuan LP, Wan JM (2014) *Days to heading 7*, a major quantitative locus determining photoperiod sensitivity and regional adaptation in rice. *Proc Natl Acad Sci U S A* 111:16337–16342
- Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. *Science* 327:812–818
- He GM, Luo XJ, Tian F, Li KG, Zhu ZF, Su W, Qian XY, Fu YC, Wang XK, Sun CQ, Yang JS (2006) Haplotype variation in structure and expression of a gene cluster associated with a quantitative trait locus for improved yield in rice. *Genome Res* 16:618–626
- Huang RY, Jiang LR, Zheng JS, Wang TS, Wang HC, Huang YM, Hong ZL (2013) Genetic bases of rice grain shape: so many genes, so little known. *Trends Plant Sci* 18:218–226
- Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, Xia G, Chu C, Li J, Fu X (2009) Natural variation at the *DEP1* locus enhances grain yield in rice. *Nat Genet* 41:494–497
- Huo X, Wu S, Zhu ZF, Liu FX, Fu YC, Cai HW, Sun XY, Gu P, Xie DX, Tan LB, Sun CQ (2017) *NOG1* increases grain production in rice. *Nat Commun* 8:1497
- Hwang I, Sheen J, Muller B (2012) Cytokinin signaling networks. *Annu Rev Plant Biol* 63:353–380
- Jiao YQ, Wang YH, Xue DW, Wang J, Yan MX, Liu GF, Dong GJ, Zeng DL, Lu ZF, Zhu XD, Qian Q, Li JY (2010) Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. *Nat Genet* 42:541–536
- Kaur A, Sidana K, Bhatia D, Neelam K, Singh G, Sahi GK, Gill BK, Sharma P, Yadav IS, Singh K (2018) A novel QTL *qSPP2.2* controlling spikelet per panicle identified from *Oryza longistaminata* (A. Chev. et Roehr.), mapped and transferred to *Oryza sativa* (L.). *Mol Breed* 38:92–104
- Liu E, Liu Y, Wu GC, Zeng SY, Thi TGT, Liang LJ, Liang YF, Dong ZY, She D, Wang H, Zaid IU, Hong DL (2016a) Identification of a candidate gene for panicle length in rice (*Oryza sativa* L.) via association and linkage analysis. *Front Plant Sci* 7:596
- Liu TM, Liu HY, Zhang H, Xing YZ (2013) Validation and characterization of *Ghd7.1*, a major quantitative trait locus with pleiotropic effects on spikelets per panicle, plant height,

- and heading date in rice (*Oryza sativa* L.). *J Integr Plant Biol* 55:917–927
- Liu TM, Mao DH, Zhang SP, Xu CG, Xing YZ (2009) Fine mapping *SPP1*, a QTL controlling the number of spikelets per panicle, to a BAC clone in rice (*Oryza sativa*). *Theor Appl Genet* 118:1509–1517
- Liu X, Zhao ZG, Liu LL, Xiao YH, Tian YL, Liu SJ, Chen LM, Wang YH, Liu YQ, Chen SH, Zhang WW, Wang CM, Jiang L, Wan JM (2016b) Construction of chromosomal segment substitution lines and genetic dissection of introgressed segments associated with yield determination in the parents of a super-hybrid rice. *Plant Breed* 135:63–72
- Lu ZF, Yu H, Xiong GS, Wang J, Jiao YQ, Liu GF, Jing YH, Meng XB, Hu XM, Qian Q, Fu XD, Wang YH, Li JY (2013) Genome-wide binding analysis of the transcription activator *IDEAL PLANT ARCHITECTURE1* reveals a complex network regulating rice plant architecture. *Plant Cell* 25:3743–3759
- Luo X, Tian F, Fu Y, Yang J, Sun C (2009) Mapping quantitative trait loci influencing panicle-related traits from Chinese common wild rice (*Oryza rufipogon*) using introgression lines. *Plant Breed* 128:559–567
- McCouch SR, CGSNL (2008) Gene nomenclature system for rice. *Rice* 1(1):72–84
- Mei HW, Xu JL, Li ZK, Yu XQ, Guo LB, Wang YP, Ying CS, Luo LJ (2006) QTLs influencing panicle size detected in two reciprocal introgressive line (IL) populations in rice (*Oryza sativa* L.). *Theor Appl Genet* 112:648–656
- Meng L, Li HH, Zhang LY, Wang JK (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J* 3:269–283
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8:4321–4325
- Qi L, Ding YB, Zheng XM, Xu R, Zhang LZ, Wang YY, Wang XN, Zhang LF, Cheng YL, Qiao WH, Yang QW (2018) Fine mapping and identification of a novel locus *qGL12.2* control grain length in wild rice (*Oryza rufipogon* Griff.). *Theor Appl Genet* 131:1497–1508
- Sasaki K, Fujita D, Koide Y, Lumanglas PD, Gannaban RB, Tagle AG, Obara M, Fukuta Y, Kobayashi N, Ishimaru T (2017) Fine mapping of a quantitative trait locus for spikelet number per panicle in a new plant type rice and evaluation of a near-isogenic line for grain productivity. *J Exp Bot* 68:2693–2702
- Sun ZZ, Yin XL, Ding J, Yu D, Hu M, Sun XW, Tan YN, Sheng XB, Liu L, Mo Y, Ouyang N, Jiang BB, Yuan GL, Duan MJ, Yuan DY, Fang J (2017) QTL analysis and dissection of panicle components in rice using advanced backcross populations derived from *Oryza Sativa* cultivars HR1128 and ‘Nipponbare’. *PLoS One* 12:e0175692
- Tang XX, Gong R, Sun WQ, Zhang CP, Yu SB (2018) Genetic dissection and validation of candidate genes for flag leaf size in rice (*Oryza sativa* L.). *Theor Appl Genet* 131:801–815
- Tian F, Zhu ZF, Zhang BS, Tan LB, Fu YC, Wang XK, Sun CQ (2006) Fine mapping of a quantitative trait locus for grain number per panicle from wild rice (*Oryza rufipogon* Griff.). *Theor Appl Genet* 113:619–629
- Venuprasad R, Dalid CO, Del Valle M, Zhao D, Espiritu M, Cruz MTS, Amante M, Kumar A, Atlin GN (2009) Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theor Appl Genet* 120:177–190
- Wang SS, Chen RK, Chen KY, Liu CY, Kao SM, Chung CL (2017) Genetic mapping of the *qSBN7* locus, a QTL controlling secondary branch number per panicle in rice. *Breed Sci* 67:340–347
- Wei XJ, Xu JF, Guo HN, Jiang L, Chen SH, Yu CY, Zhou ZL, Hu PS, Zhai HQ, Wan JM (2010) *DTH8* suppresses flowering in rice, influencing plant height and yield potential simultaneously. *Plant Physiol* 153:1747–1758
- Weng XY, Wang L, Wang J, Hu Y, Du H, Xu CG, Xing YZ, Li XH, Xiao JH, Zhang QF (2014) Grain number, plant height, and heading Date7 is a central regulator of growth, development, and stress response. *Plant Physiol* 164:735–747
- Wu SJ, Zhong H, Zhou Y, Zuo H, Zhou LH, Zhu JY, Ji CQ, Gu SL, Gu MH, Liang GH (2009) Identification of QTLs for the resistance to rice stripe virus in the *indica* rice variety Dular. *Euphytica* 165:557–565
- Wu Y, Wang Y, Mi XF, Shan JX, Li XM, Xu JL, Lin HX (2016) The QTL *GNP1* encodes GA20ox1, which increases grain number and yield by increasing cytokinin activity in rice panicle meristems. *PLoS Genet* 12:e1006386
- Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang QF (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theor Appl Genet* 105:248–257
- Xing YZ, Tang WJ, Xue WY, Xu CG, Zhang QF (2008) Fine mapping of a major quantitative trait loci, *qSSP7*, controlling the number of spikelets per panicle as a single Mendelian factor in rice. *Theor Appl Genet* 116:789–796
- Xu ZP, Li SC, Zhang CQ, Zhang BC, Zhu KZ, Zhou YH, Liu QQ (2017) Genetic connection between cell-wall composition and grain yield via parallel QTL analysis in *indica* and *japonica* subspecies. *Sci Rep* 7:12561
- Xue WY, Xing YZ, Weng XY, Zhao Y, Tang WJ, Wang L, Zhou HJ, Yu SB, Xu CG, Li XH, Zhang QF (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nat Genet* 40:761–767
- Yan WH, Wang P, Chen HX, Zhou HJ, Li QP, Wang CR, Ding ZH, Zhang YS, Yu SB, Xing YZ, Zhang QF (2011) A major QTL, *Ghd8*, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. *Mol Plant* 4:319–330
- Yan CJ, Yan S, Yang YC, Zeng XH, Fang YW, Zeng SY, Tian CY, Sun YW, Tang SZ, Gu MH (2009) Development of gene-tagged markers for quantitative trait loci underlying rice yield components. *Euphytica* 169:215–226
- Yu HP, Qiu ZN, Xu QK, Wang ZW, Zeng DL, Hu J, Zhang GH, Zhu L, Gao ZY, Chen G, Guo LB, Qian Q, Ren DY (2017) Fine mapping of *LOW TILLER 1*, a gene controlling tillering and panicle branching in rice. *Plant Growth Regul* 83:93–104
- Zhang H, Zhao Q, Sun ZZ, Zhang CQ, Feng Q, Tang SZ, Liang GH, Gu MH, Han B, Liu QQ (2011) Development and high-throughput genotyping of substitution lines carrying the chromosome segments of *indica* 9311 in the background of *japonica* Nipponbare. *J Genet Genomics* 38:603–611
- Zhou LJ, Chen LM, Jiang L, Zhang WW, Liu LL, Liu X, Zhao ZG, Liu SJ, Zhang LJ, Wang JK, Wan JM (2009) Fine mapping of the grain chalkiness QTL *qPGWC-7* in rice (*Oryza sativa* L.). *Theor Appl Genet* 118:581–590

Zhou Y, Tao Y, Yuan Y, Zhang Y, Miao J, Zhang R, Yi C, Gong Z, Yang Z, Liang G (2018) Characterisation of a novel quantitative trait locus, *GN4-1*, for grain number and yield in rice (*Oryza sativa* L.). *Theor Appl Genet* 131:637–648

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.