RESEARCH PAPER

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

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Abstract

Total spikelet number per panicle (TSN) is one of the determinants of grain productivity in rice (Oryza sativa L.). In this study, we attempted to detect quantitative trait loci (QTLs) for TSN in the introgression lines with high TSN, derived from the cross of Indica Group variety IR 64 with new plant type lines. Two QTLs were detected on the long arm of chromosome 12: qTSN12.1 in the BC2F2 population of YTH63/IR 64 and qTSN12.2 in the BC4F3 population of YTH83/IR 64. TSN of the main tiller was significantly higher in near-isogenic lines (NILs) for qTSN12.1 (IR 64-NIL1; 188.6) and for qTSN12.2 (IR 64-NIL12; 199.4) than in IR 64 (141.2), owing to a significant increase in both primary and secondary branch numbers. These results suggest the critical function of these QTLs in the promotion of rachis branching at the panicle formation stage. Fine mapping of qTSN12.2 revealed six candidate genes in a 92-kb region of the Nipponbare reference genome sequence between flanking markers RM28746 and RM28753. Detailed phenotyping of agronomic traits of IR 64-NIL12 carrying qTSN12.2 showed drastic changes in plant architecture: this line had lower panicle number, longer culm, and longer and wider leaves compared with IR 64. Percentage of fertility and 1000-grain weight tended to be greater, and grain yield per square meter was also greater in IR 64-NIL12 than in IR 64. The newly identified QTLs will be useful for genetic improvement of the yield potential of Indica Group varieties. The markers tightly linked to qTSN12.2 are available for marker-assisted breeding.

Key words: Grain yield, Indica Group, near-isogenic line, Oryza sativa L., quantitative trait locus, total spikelet number per panicle, yield component.
Introduction

Rice (*Oryza sativa* L.) is an important crop that serves as the staple food of more than half the world’s population. To satisfy the increasing global demand of the growing population, a 50% increase in rice production will be required by the year 2050 (Alexandratos and Bruinsma, 2012), especially in developing countries of Asia and Africa, where populations have been increasing dramatically (Seck et al., 2012). Of the two main subspecies of cultivated rice, the Indica Group varieties are grown predominantly in southern China, Southeast Asia, and South Asia, occupying about 70% of the world’s rice-producing area. The Japonica Group varieties, on the other hand, are grown mainly in East Asia (Zhao et al., 2011; Huang et al., 2012). Considering their vast area of production, the development of Indica Group varieties with high yield potential will contribute to global food security.

Rice yield is characterized by total spikelet number per panicle (TSN), panicle number, fertility, and 1000-grain weight. TSN is a key factor directly associated with rice productivity; hence, many studies have been conducted to identify genetic factors for TSN and elucidate its contribution to genetic improvement of yield potential (see review by Bai et al., 2012). Quantitative trait loci (QTLs) for TSN in rice have been identified in various segregating populations (Bai et al., 2012). In addition to QTLs for TSN, QTLs for controlling panicle architecture, such as the number of primary and secondary branches and spikelet number per primary and secondary branch, have also been mapped and studied (Yamagishi et al., 2002; Ando et al., 2008). Three of the QTLs associated with both TSN and panicle architecture, namely qTSN4.4*SPIKEELSLCH4 (Fujita et al., 2012, 2013; Zhang et al., 2014), SCM2*APO1 (Ookawa et al., 2010; Terao et al., 2010), and DEP1 (Huang et al., 2009), were found to increase grain yield in near-isogenic lines (NILs) through drastic changes in plant architecture under field conditions.

In the late 1980s, a breeding program to develop new plant type (NPT) lines was launched at the International Rice Research Institute (IRRI) with the aim of increasing the yield potential of Indica Group inbred varieties in a tropical environment. The ideotypes of NPT would have low tiller number, few unproductive tillers, 200–250 grains per panicle, and increased harvest index (Khush, 1995). The IRRI breeding programs used Tropical Japonica Group varieties as donors; these varieties had low tillering, few unproductive tillers, large panicles, thick culms, lodging resistance, and large dark green flag leaves (Khush, 1995). The developed NPT lines had higher TSN and 1000-grain weight, and a lower number of unproductive tillers; however, the NPT lines did not yield more grain because of poor fertility and fewer panicles (Peng et al., 2008).

In the 1990s, a breeding objective was set under the IRRI-Japan Collaborative Research Project to improve the yield potential of the Indica Group variety IR 64 by using NPT lines and a Japanese high-yielding variety, Hoshiaoba, as donor parents. A total of 334 introgression lines (ILs, BC3-derived lines) with unique agronomic traits (high TSN, earlier or later heading date, and greater 1000-grain weight, leaf size, and culm length) were developed in the IR 64 genetic background by recurrent backcross breeding (Fujita et al., 2009, 2010). Fujita et al. (2009) used these ILs to map chromosomal regions that were associated with favorable agronomic traits, including TSN. Using BC3F2 populations derived from the crosses between the ILs and IR 64, six QTLs for TSN were detected: five on chromosome 4, qTSN4.1–qTSN4.5 (Fujita et al., 2012), and one on chromosome 7, qTSN7.1 (Koide et al., 2013). The allelic effects of these QTLs were confirmed in NILs. Among the 334 ILs, a few lines had considerably high TSN but lacked segments containing qTSN4s and qTSN7.1 (Fujita et al., 2010). Therefore, we hypothesized that as yet unidentified QTLs for TSN are present on other chromosomes.

This study was conducted to explore new QTLs for TSN in two ILs with high TSN, YTH63 and YTH83, selected from among the 334 ILs. Two QTLs for TSN were identified and one of them was fine-mapped. NILs carrying QTLs for TSN were developed and used to investigate grain yield, yield components, and other agronomic traits under field conditions.

Table 1. Plant materials in this study

| Entry no. | IRRI accession no. | Entry no. | IRRI accession no. | Parental varieties of donor | Chromosomal location of introgressed segments |
|-----------|--------------------|-----------|--------------------|-----------------------------|---------------------------------------------|
| YTH63c    | IR 84635-10-59-4-2-2-3-4-2-2-8-B | YP3       | IR 65598-112-2     | Shen Nung 89–366, Genjah Wangkal NO 11, Bali Ontjer | 1S, 12L                                      |
| YTH83c    | IR 84642-8-4-3-4-4-2-4-2-6-B    | YP4       | IR 65564-2-2-3     |                             | 1S, 1L, 4L, 12L                             |

a Donors are NPT lines bred at IRRI.
b Pedigrees obtained from the International Rice Information System (IRIS; http://www.iris.irri.org/). Bold indicate varieties from Indonesia (Tropical Japonica Group).
c Information from Fujita et al. (2010). Refer to Supplementary Fig. S1 for graphical genotype.
the short arm of chromosome 1 and long arm of chromosome 12 (Table 1 and Supplementary Fig. S1). YTH63 and YTH83 were crossed with IR 64 to generate BC$_{F_2}$ populations for QTL analysis (Supplementary Fig. S2). In the BC$_{F_2}$ population of YTH63 and IR 64, one QTL peak was found on chromosome 4 and another one on chromosome 12. To clarify the effect of the QTL on chromosome 12, we selected a BC$_{F_2}$ plant that was heterozygous on chromosome 12 and IR 64-fixed homozygous on chromosome 4, and a total of 201 BC$_{F_2}$ plants were generated by selfing from the BC$_{F_2}$F$_2$ plants and used for QTL analysis (see Supplementary Fig. S2).

Plant growth conditions and panicle sampling
IR 64, YTH63, YTH83 and 185 BC$_{F_2}$ plants of YTH63/IR 64 and 201 BC$_{F_2}$F$_2$ plants of YTH63/IR 64 were grown in an experimental paddy field in IRRI, Los Baños, the Philippines (14°11′N, 121°15′E). Soaked seeds were sown in trays containing sterilized fine soil and placed in a greenhouse. At 21 days after sowing, seedlings (one plant per hill) were transplanted at 20 cm between hills and 30 cm between rows. Basal fertilizer (30 kg ha$^{-1}$ each of N, P$_2$O$_5$, and K$_2$O) was applied as dressing before transplanting. At 2 and 4 weeks after transplanting, ammonium sulfate was applied at 30 kg N ha$^{-1}$ as top dressing.

In the BC$_{F_2}$ population (YTH63/IR 64), panicles of the three tallest culms of each individual plant were collected for counting TSN; the average TSN values were used for segregation analysis. In the BC$_{F_2}$F$_2$ population (YTH83/IR 64), the panicles of the tallest culm were harvested for counting TSN.

QTL analysis
Genomic DNA from individual plants in each of the BC$_{F_2}$ and BC$_{F_2}$F$_2$ populations was extracted from freeze-dried leaves using the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich, 1985). The DNA samples were analyzed with simple sequence repeat (SSR) markers corresponding to known introgressed regions in each IL (Fujita et al., 2010; McCouch et al., 2002). PCR amplification and electrophoresis were conducted by the method described by Fujita et al. (2012). A linkage map with SSR markers located on all introgression segments was constructed based on genotypes of BC$_{F_2}$F$_2$ individuals in each population by using the Kosambi function (Kosambi, 1943). Composite interval mapping was performed using Windows QTL Cartographer V2.5 (Wang et al., 2010). The proportion of observed phenotypic variations attributable to a particular chromosomal region was estimated by the coefficient of determination ($R^2$). The critical threshold values of the LOD score for QTL identification were calculated by conducting 1000 permutation tests with significance at $P<0.05$.

Substitution mapping
The development scheme for plant materials and populations used for QTL analysis and substituting mapping in this study are shown in Supplementary Fig. S2. Seven recombinants between RM28621 and RM1226 were selected from BC$_{F_2}$ plants for the first substitution mapping. A total of 24 BC$_{F_2}$F$_2$ progeny from each selected BC$_{F_2}$F$_2$ plant (named HFO entry) were grown. At least three homozygous recombinant plants in each BC$_{F_2}$F$_2$ progeny were selected for TSN evaluation and estimation of the genotype for the QTL mapping. Primers designed using the genome sequence of Nipponbare (Rice Genome Research Program; http://rapdb.dna.affrc.go.jp/) are listed in Supplementary Table S1. Using these primers, additional recombinant BC$_{F_2}$F$_2$ plants from a large population (5841 plants) were selected. For the second substitution mapping, a total of 47 recombinants between KM12016 and RM17 (named MBV entry) were selected to narrow down the candidate QTL region. Seling of these selected plants produced homozygous recombinant BC$_{F_2}$F$_2$ plants. To determine the genotype for the QTL in the BC$_{F_2}$F$_2$ recombinants, 24 BC$_{F_2}$F$_2$ progeny and IR 64 were grown in the paddy field. Plant growth conditions both for the first and second substitution mapping were the same as described in ‘Plant growth conditions and panicle sampling’. One panicle was harvested per plant from the tallest culm for TSN counting.

NIL development
One plant containing the detected QTL for TSN and the smallest number of introgressed segments was selected from the BC$_{F_2}$F$_2$ (YTH63/IR 64) population, and was used to generate BC$_{F_3}$F$_2$ lines by self-pollination. A NIL selected from the BC$_{F_2}$F$_2$ lines was designated IR 64-NIL1. One plant was selected from the BC$_{F_3}$F$_3$ (YTH83/IR 64) population with the same method and designated IR 64-NIL12. (IR 64-NILs were numbered in chronological order as they were developed under the IRRI-Japan Collaborative Research Project.) The NILs were grown during the dry season (DS) of 2009 in IRRI. One panicle in the tallest culm of each of at least nine plants was collected for counting the numbers of primary branches, secondary branches, tertiary branches, number of spikelets on each branch, and TSN. The numbers of primary, secondary, and tertiary branches were counted according to the rice panicle structure described by Ikeda et al. (2010).

Gene expression analysis and sequencing of a candidate gene
Quantitative RT-PCR (qRT-PCR) for annotated genes located in the region of $qTSN12.2$ was performed. IR 64 and IR 64-NIL12 were grown in an experimental paddy field of NICS, Ibaraki, Japan (36°00′N, 140°01′E) in 2016. Note that increased TSN in IR 64-NIL12 was stably observed also in this experimental paddy field (see Supplementary Table S2). Three developing panicles (2–5 mm) were collected as one biological replicate from a plant at panicle initiation stage, and four biological replicates (four plants) were prepared for this expression analyses in both genotypes. Total RNA was extracted by using a RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). One microgram of total RNA was used for the reverse transcriptase reaction (Prime Script RTase, Takara Bio Inc., Otsu, Japan). qRT-PCR reactions were carried out with 1.5 μl cDNA mixtures under a Thermal Cycler Dice Real Time System III (Takara Bio). The expression level of annotated genes was normalized to the expression of a house- hold gene, ubiquitin (Os01g22490), in the developing panicles (Fujita et al., 2013). The expression level of each gene was compared between IR 64 and IR 64-NIL12 using the ΔΔ$C_{t}$ method (Livak and Schmittgen, 2001). All statistics were performed at the Δ$C_{t}$ stage with the t-test.

Grain yield, shoot biomass, and harvest index
IR 64 and IR 64-NIL12 were cultivated for yield measurements in the wet season (WS) of 2011 and 2012, and in the 2012 DS and 2013 DS in IRRI. Three germinated seeds were sown into each cell of a cell tray and grown in the greenhouse under natural light conditions for 3 weeks. Seedlings (three per hill) were transplanted at 20 cm between hills and 25 cm between rows. The area of each plot was 4.8 m$^2$, with three or four replications. The experimental plots were arranged in a complete randomized block design. Basal and top dressings were applied as described above. At maturity, 20 plants corresponding to 1 m$^2$ were harvested at the soil surface in each plot. Grain and straw were dried in a greenhouse. Grain yield was calculated on a 14% moisture content basis.

Yield components and agronomic traits
Yield components and agronomic traits in IR 64 and IR 64-NIL12 were measured in 2011 WS and 2012 DS in IRRI. Days to heading was determined as the number of days from seeding to 50% flowering. Panicle number, TSN, percentage fertility, and 1000-grain weight were determined at maturity from five plants

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selected from each plot of the yield measurements. After being removed from panicles, approximately 30 g of grains was randomly selected as subsamples to determine total spikelet number per square meter, fertility, and 1000-grain weight. Sterile and fertile grains were manually selected and 1000-grain weight of fertile grain was calculated on a 14% moisture content basis. Culm length, panicle length, leaf length, and leaf width were measured on the tallest tiller of each plant, with culm length measured from the soil surface to the panicle neck, panicle length measured from the panicle neck to the panicle tip, and leaf width and leaf length measured on the second leaf (i.e. the one below the flag leaf).

Results

QTL analysis for TSN

TSN of YTH63 was $148.3 \pm 19.3$ (mean±standard deviation), which was considerably higher than that of IR 64 ($98.2 \pm 14.4$) (Fig. 1A). TSN in the BC$_4$F$_2$ (YTH63/IR 64) population showed a continuous frequency distribution and ranged from 87.7 to 202.3 (Fig. 1A). Composite interval mapping identified a QTL in YTH63 between RM6411 and RM28746 on the long arm of chromosome 12 (Fig. 2A); this QTL, designated as $qTSN12.1$, explained 10.0% of phenotypic variation (Table 2). The donor allele at $qTSN12.1$ increased TSN (Table 2). TSN of YTH83 was $177.2 \pm 41.6$, which was considerably higher than that of IR 64 ($121.1 \pm 17.2$) (Fig. 1B). TSN in the BC$_4$F$_3$ (YTH83/IR 64) population showed a continuous frequency distribution and ranged from 76 to 209 (Fig. 1B). The QTL in YTH83 was between RM28759 and RM28767 on the long arm of chromosome 12 (Fig. 2A) and was designated as $qTSN12.2$; it explained 7.0% of phenotypic variation (Table 2). The donor allele at $qTSN12.2$ increased TSN (Table 2).

Delimitation of a candidate genomic region for $qTSN12.2$

Among 201 BC$_4$F$_3$ plants (YTH83/IR 64), six that had recombination events between RM1194 and RM1227 were selected (Fig. 2B). The TSN values of HFO5, 6 and 12 were significantly higher than that of IR 64, whereas the TSN values of HFO8, 9 and 13 were slightly but not significantly higher than that of IR 64 (Fig. 2B). $qTSN12.2$ was mapped within the interval between RM28746 and RM28759 as a single Mendelian factor (Fig. 2B). For more precise determination of the position of $qTSN12.2$, we selected additional recombinant BC$_4$F$_3$ plants from a large population. The TSN values of MBV198, 177, 205, 99, 187, 69, and 79 were similar to that of IR 64, whereas the TSN values

![Fig. 1.](https://academic.oup.com/jxb/article-abstract/68/11/2693/3860984) Frequency distribution of total spikelet number per panicle in BC$_4$F$_2$ and BC$_4$F$_3$ populations derived from crosses of IR 64 with (A) YTH63 or (B) YTH83.
Fig. 2. Mapping of QTL for total spikelet number per panicle (TSN) detected on chromosome 12 in the BC4F2 population of YTH63/IR 64 (qTSN12.1) and the BC4F3 population of YTH83/IR 64 (qTSN12.2) and the location of qTSN12.2. (A) QTL analysis. Black bars indicate QTL regions. Arrowheads indicate the peak position of each QTL. (B, C) The first (B) and the second (C) substitution mapping. Graphical genotypes and total spikelet number per panicle (TSN) of the BC4F3 plants (YTH83/IR 64). Black, homozygous for YTH83; white, homozygous for IR 64; hatched, recombination regions. Positions of DNA markers are indicated by vertical lines. The TSN data are mean values with error bars indicating standard errors. Double-headed arrow indicates the qTSN12.2 candidate region.

Table 2. Quantitative trait loci (QTL) for total spikelet number in two populations

| Cross combination | Donor | QTL     | Marker interval | Chr. | LOD score | R² | Additive effect | Dominant effect |
|-------------------|-------|---------|-----------------|------|-----------|----|----------------|----------------|
| YTH63/IR 64      | YP3   | qTSN12.1| RM6411–RM28746  | 12   | 4.2       | 0.10| –9.1           | 8.5            |
| YTH83/IR 64      | YP4   | qTSN12.2| RM28759–RM28767 | 12   | 3.1       | 0.07| –10.1          | 6.2            |

*Bold indicates nearest marker to QTLs.*
*Critical threshold value of LOD score was equivalent to LOD at an experiment-wise significant level of 0.05.*
*Percentage of explained phenotypic variation.*
*Positive value indicates the direction of the effect of IR 64 allele.*
of MBV101, 139, 68, 21, and 135 were significantly higher than that of IR 64. These results strongly indicate that qTSN12.2 is located in the interval between RM28746 and RM28753. According to the Nipponbare genome sequence in the Rice Annotation Project Database (2015), the physical distance of this interval is approximately 92 kb (Fig. 2C). Furthermore, quantitative gene expression analysis was performed for open reading frames (ORFs) located in this candidate region using primer pairs described in Table 3. Of nine putative ORFs, primer pairs for Os12g0619100 and Os12g0620100 could not be developed possibly due to the negligible expression level in the various rice organs, according to the RNA-seq analysis in the Rice Annotation Project Data Base (RAP-DB, 2015). Expression of Os12g0619700 was not detectable in RT-PCR analysis. Among the remaining six putative ORFs, no gene was significantly higher in IR 64-NIL12 than IR 64 (Table 3), although expression of Os12g0619000 was up-regulated by 27% in IR 64-NIL12 relative to IR 64 (Table 3).

Development of NILs and characterization of panicle architecture

A BC₄F₃ line generated by selfing of the selected BC₄F₂ plants (YTH63/IR 64) only contained the region of qTSN12.1. This line was selected as a NIL for qTSN12.1 and designated as IR 64-NIL1 (Fig. 3A). From the BC₄F₃ population of YTH83/IR 64, one BC₄F₃ plant containing the region of qTSN12.2 was selected through the same method used for the development of IR 64-NIL1. A BC₄F₃ line generated by selfing of the selected BC₄F₃ plant was designated as IR 64-NIL12 (Fig. 3B). A chromosome segment of 3.96–6.28 Mbp from YP3 was carried on chromosome 12 of IR 64-NIL1; 3.96–4.05 Mbp of chromosome segment from YP4 was carried on chromosome 12 of IR 64-NIL12. In both cases, the interval of chromosome segment from donor parent is between RM1102 and RM1227.

To confirm the effects of qTSN12.1 and qTSN12.2 on TSN, spikelets were counted separately on the primary and secondary branches of the NILs. The TSN was 188.6 ± 6.8 in IR 64-NIL1 and 199.4 ± 9.1 in IR 64-NIL12, which were significantly higher than that of IR 64 (141.2 ± 5.6) (Table 4). The spikelet numbers on the primary rachis were 53.7 ± 1.2 in IR 64-NIL1 and 50.7 ± 1.6 in IR 64-NIL12, which were significantly higher than that of IR 64 (45.6 ± 1.0). The spikelet numbers on the secondary rachis were 134.9 ± 6.3 in IR 64-NIL1 and 148.7 ± 9.1 in IR 64-NIL12, which were also significantly higher than that of IR 64 (95.6 ± 5.3) (Table 4). The numbers of primary and secondary branches in the two NILs were also significantly higher than that of IR 64 (Table 4). The average spikelet number per branch located on the secondary rachis of the NILs was significantly higher than that of IR 64, but those on primary branches of the NILs and IR 64 were statistically similar (see Supplementary Table S3).

Grain yield, shoot biomass, and harvest index

IR 64-NIL12 was selected for yield measurements because it had a smaller segment introgressed from the donor parent on the long arm of chromosome 12 than in IR 64-NIL1. Grain

### Table 3. Relative expression and primer information on annotated genes in candidate region of qTSN12.2

| Locus | Annotation | Forward primer sequence (5′–3′) | Reverse primer sequence (5′–3′) | Relative expression (IR 64-NIL12/IR 64) |
|-------|------------|-------------------------------|-------------------------------|---------------------------------------|
| Os12g0618800 | Protein of unknown function DUF266, plant family protein | GCGAGCAGTTTGTTCACTCA | GGGATCGGATCTTGCTTACA | 1.10 ns |
| Os12g0619000 | IQ calmodulin-binding region domain containing protein | ATCAGTGGAGCCAGAATTGG | GGCCTCATTTTCATCAGCAT | 1.27 ns |
| Os12g0619700 | Conserved hypothetical protein | AACCTGTTTACTGCGGTTCG | AATGTCCCTGCAGAACCTTG | nd |
| Os12g0620000 | Similar to leucine rich repeat family protein | AGAAGCATCCGCAGCTATGT | CGCCACTCTGAGAACTGACA | 1.15 ns |
| Os12g0620400 | Methyl-CpG DNA binding domain containing protein | GTCTCGTCTCTTCTCTCTCCATTTT | CATGGTTTCGAGTTTTCGCTTC | 0.91 ns |
| Os12g0620600 | Conserved hypothetical protein | TTGATTCTGGGTACCGCTTC | CCTAATTCCACCAGGCTCAA | 0.82 ns |
| Os12g0621000 | Similar to ubiquitin carboxyl-terminal hydrolase | CATTTGGGGATTTGTTGAGG | AGCTCCTGGGAATCATGTTG | 0.90 ns |

*Os12g0619100 (heat shock protein DnaJ, cysteine-rich domain containing protein) and Os12g0620100 (zinc finger, RING-type domain containing protein) are also annotated in this region.

**ns**. the differences were not significant; **nd**, gene expression was not detected.
yield per square meter ranged from 380 to 508 g m⁻² in IR 64 and from 512 to 598 g m⁻² in IR 64-NIL12 (Fig. 4A). Grain yield per square meter was higher in IR 64-NIL12 by 18–36% than in IR 64, and this difference was significant for three of the four seasons (Fig. 4A). Shoot biomass was similar between IR 64 and IR 64-NIL12 in three of the four seasons (Fig. 4B). Harvest index of IR 64-NIL12 was significantly higher (by 11–32%) than that of IR 64 in all four seasons (Fig. 4C).

**Yield components and agronomic traits**

Agronomic traits (days to heading, culm length, panicle length, leaf length, and leaf width) measured in 2011 WS and 2012 DS are shown in Table 5. IR 64-NIL12 had longer culms and longer and wider leaves than IR 64 in both seasons. There were no significant differences in days to heading. These results indicate that the region of qTSN12.2 increases plant height and leaf size without any changes in days to heading. Panicle length was similar between genotypes.

Yield components of IR 64 and IR 64-NIL12 were evaluated in the 2011 WS and 2012 DS (Table 6). TSN values in IR 64-NIL12 were significantly higher by 20% (2011 WS) and 75% (2011 WS) than that of IR 64. Panicle numbers in IR 64-NIL12 were significantly lower by 16% (2011 WS) and 26% (2012 DS) than those of IR 64. Tiller numbers of IR 64-NIL12 were also significantly lower than those of IR 64 in the period of most growth in both DS and WS (Supplementary Fig. S3). Fertility of IR 64-NIL12 was 9% (2011 WS) and 6% higher (2012 DS) than that of IR 64 and 1000-grain weights were 3% and 5% greater (Table 6); however, these differences were significant only in the DS.

**Discussion**

Detection of QTLs for TSN, qTSN12.1 and qTSN12.2, on chromosome 12

We previously detected QTLs for TSN on chromosome 4 (qTSN4.1–qTSN4.5, Fujita et al., 2012) and chromosome 7 (qTSN7.1, Koide et al., 2013). In this study, we hypothesized that undetected QTLs for TSN derived from NPT lines are also present on other chromosomes. Using populations derived from crosses between IR 64 and YTH63 and between IR 64 and YTH83, two such QTLs, qTSN12.1 and qTSN12.2, were detected on the long arm of chromosome 12 (Fig. 2A and Table 2). In close proximity, qGPP12, a QTL associated with spikelet number, was detected in a population derived...
from a cross between Zhenshan 97 (Indica Group) and Teqing (Indica Group), in which the Teqing allele increased TSN (Liu et al., 2011). Subsequently, we succeeded in developing two NILs, IR 64-NIL1 carrying qTSN12.1 and IR 64-NIL12 carrying qTSN12.2, with IR 64 genetic backgrounds in this study (Fig. 3). In both NILs, TSNs of the main tiller were significantly higher than that of IR 64 (Table 4). The numbers of spikelets on both primary and secondary rachis branches were significantly higher in IR 64-NIL1 and IR 64-NIL12 than in IR 64 (Table 4), whereas the average spikelet number per branch in the NILs was similar to that of IR 64 (see Supplementary Table S3). Therefore, the greater TSN in the NILs was attributed to greater branching. It is concluded that the donor alleles of qTSN12.1 and qTSN12.2 promote branching in the primary and secondary rachis in the genetic background of IR 64.

We further fine-mapped qTSN12.2 in the interval between RM28746 and RM28753 (Fig. 2B, C). Physical distance of the candidate region for qTSN12.2 is 92-kb according to the Nipponbare reference genome sequence (Fig. 2C). This region contains nine putative ORFs. Among predicted genes in the candidate region of qTSN12.2, no gene showed significant difference in expression level between IR 64 and IR 64-NIL12, but our survey and analysis on gene expression narrowed down the candidate genes to six (Table 3). There is no report so far demonstrating a homologue of any of the listed six genes was involved in increase in TSN. The causal gene for qTSN12.2 is possibly the novel gene controlling the TSN. Further genetic studies sequencing the six candidate genes to find polymorphisms and subsequent validation with transgenic plants are required to determine the causal gene for qTSN12.2.

Drastic changes in plant architecture in IR 64-NIL12 carrying qTSN12.2

Under field conditions, IR 64-NIL12 showed not only higher TSN, but also significantly lower tiller numbers, longer culms and leaves, and wider leaves compared with IR 64 (Table 5 and Supplementary Fig. S3). Reduced panicle number at maturity (Table 6) was in agreement with a lower tiller number (Supplementary Fig. S3). These results imply that the introgression of the qTSN12.2 region drastically changes agronomic traits in the above-ground organs of IR 64. qGPP12 increases TSN through prolonged growth under long-day conditions (Liu et al., 2011). Although the effects of qTSN12.2 and qGPP12 on TSN are similar (as mentioned in the previous subsection), the effects of these QTLs on growth duration seem to be different because number of days to heading was not affected by qTSN12.2 (Table 5). QTLs for tiller (panicle) number were detected close to the qTSN12.2 locus (Yan et al., 1999; Liao et al., 2001; Hittalmani et al., 2003; Yang et al., 2006; Liu et al., 2012). A QTL for leaf width was also identified near this region (Yan et al., 1999). There is a possibility that qTSN12.2 could play a crucial role in modifying plant architecture both in sink organs (TSN and tiller (panicle) number) and source organs (leaf size). Further genetic study is needed to clarify whether agronomic traits pleiotropically changed in IR 64-NIL12 are under the control of a single causal gene or multiple genes independently in the region of qTNS12.2.

Genetic improvement of yield potential in Indica Group varieties with the introgression of qTSN12.2 region from a New Plant Type variety

Grain yield per square meter was significantly higher in IR 64-NIL12 than in IR 64 in three of the four seasons...
Table 5. Agronomic traits for IR 64 and near-isogenic line IR 64-NIL12 in the wet season of 2011 and dry season of 2012

| Season | Line     | Days to heading | Culm length (cm) | Panicle length (cm) | Leaf length (cm) | Leaf width (cm) |
|--------|----------|-----------------|------------------|---------------------|-----------------|----------------|
| 2011   | Wet      | IR 64           | 84.8 ± 0.5       | 77.9 ± 1.7          | 25.1 ± 0.2      | 38.7 ± 0.9     |
|        |          | IR 64-NIL12     | 85.3 ± 0.5 ns    | 81.3 ± 0.8 ns       | 26.1 ± 0.5 ns   | 45.2 ± 1.0**  |
| 2012   | Dry      | IR 64           | 82.8 ± 0.3       | 65.4 ± 0.4          | 23.2 ± 0.5      | 34.8 ± 0.9     |
|        |          | IR 64-NIL12     | 82.5 ± 0.3 ns    | 72.3 ± 1.0**        | 23.9 ± 0.3 ns   | 39.3 ± 0.5**  |

Values are means ± standard error. * or ** indicate that IR 64-NIL12 differed significantly from IR 64 at the 5% or 1% level, respectively (t-test). ns, difference not significant.

Table 6. Yield components for IR 64 and near-isogenic line (IR 64-NIL12) in the wet season of 2011 and the dry season of 2012

| Season | Line     | Panicle no. (plant⁻¹) | Panicle no. (m⁻²) | Total spikelet no. (plant⁻¹) | Total spikelet no. (m⁻²) | Fertility (%) | 1000-grain weight (g) |
|--------|----------|------------------------|-------------------|-----------------------------|-------------------------|--------------|----------------------|
| 2011   | Wet      | IR 64                  | 16.7 ± 0.7        | 333.5 ± 14.7                | 68.4 ± 4.5              | 74.8 ± 2.7   | 26.5 ± 0.2           |
|        |          | IR 64-NIL12            | 14.0 ± 0.6 (0.84)*| 280.0 ± 11.2 (0.84)*        | 82.3 ± 2.9 (1.20)*      | 23055 ± 1337 (1.01) ns | 81.6 ± 1.5 (1.09) ns | 27.2 ± 0.3 (1.03) ns |
| 2012   | Dry      | IR 64                  | 21.1 ± 0.8        | 422.0 ± 16.2               | 46.9 ± 2.4              | 19860 ± 1519 | 84.1 ± 1.0           |
|        |          | IR 64-NIL12            | 15.7 ± 0.7 (0.74)*| 314.0 ± 14.1 (0.74)*       | 82.0 ± 2.5 (1.75)**     | 25765 ± 1425 (1.30)** | 89.4 ± 0.9 (1.06)**  | 27.8 ± 0.3 (1.05)*  |

The values are mean ± standard error (proportion of the value for IR 64). * or ** indicate significant difference with IR 64 at the 5% or 1% level according to the t-test. ns, difference not significant.

(Fig. 4A). The TSN was also higher in IR 64-NIL12 than in IR 64 both in the 2011 WS and 2012 DS (Table 6), consistent with the results for TSN of the main stem (Table 4). These results indicate that the enhanced TSN by the donor allele of qTSN12.2 is one of the critical factors for increasing grain yield in the IR 64 genetic background. Fertility and 1000-grain weight in the IR 64-NIL12 were higher in both 2011 WS and 2012 DS (Table 6), and significantly so in 2012 DS (Fig. 4A). The TSN was also higher in IR 64-NIL12 than in IR 64 (Fig. 4A). The developed markers linked with qTSN12.2 could be useful for the genetic improvement of yield potential of Indica Group varieties. Note, however, that panicle number was reduced due to the introgression of the qTSN12.2 region (Table 6). Khush et al. (1995) described that high TSN is often associated with low panicle number. IR 64-NIL12 is not an exception to this trade-off relationship. To further support the genetic improvement in the yield potential of Indica Group varieties and to recover the reduction in panicle number observed in IR 64-NIL12 carrying qTSN12.2, introgression of positive QTLs for panicle number, located at a different locus from qTSN12.2, into IR 64-NIL12 is required as a genetic approach. The establishment of better fertilizer management to promote tillering in IR 64-NIL12 could be an effective agronomic approach.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Graphical genotypes of the introgression lines YTH63 and YTH83.

Fig. S2. Scheme of development for mapping populations and near-isogenic lines.

Fig. S3. Tiller numbers for IR 64 and IR 64-NIL12 grown in a paddy field in the wet season of 2011 and dry season of 2012.

Table S1. Molecular markers developed for substitution mapping.

Table S2. Total spikelet number per panicle (TSN) for IR 64 and IR 64-NIL12 in the experimental field of NICS, Japan, in 2011 and 2012.

Table S3. Number of spikelets on primary and secondary branches in IR 64 and near-isogenic lines carrying qTSN12 in dry season of 2009.
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