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# Characterization of QTL for unique agronomic traits of new-plant-type rice varieties using introgression lines of IR64

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## ARTICLE INFO

## ABSTRACT

To enhance the yield potential of an elite *indica* rice cultivar, an introgression (BC₃-derived) line of IR64, YTH288, was developed using a new-plant-type cultivar, IR66215-44-2-3, as a donor parent. YTH288 has agronomically valuable characteristics such as large panicles, few unproductive tillers, and large leaves inherited from NPT. To identify the genetic basis of these traits, we used 167 $F_2$ plants derived from a cross between IR64 and YTH288 to conduct QTL analysis for five agronomic traits: days to heading (DTH), culm length (CL), flag leaf length (FLL), flag leaf width (FLW), and filled spikelet number per panicle (FSN). Six putative QTL were detected: four on chromosome 4 (for CL, FLL, FLW, and FSN) and two on chromosome 2 (for DTH and FLL). All QTL with the IR66215-44-2-3 allele, except that for FLL on chromosome 2, had positive effects on each trait. To confirm the effects of these putative QTL, we developed NILs with the IR64 genetic background by marker-assisted selection. We observed significant differences in several agronomic traits between IR64 and NILs that carried these QTL on chromosomes 2 and 4. Additionally, four IR64-NILs carrying chromosomal segments derived from different NPT varieties on the long arm of chromosome 4 exhibited similar pleiotropic

effects for unique agronomic traits. These NILs can be used as research materials for studying each trait and as breeding materials for yield improvement of *indica* rice cultivars.

# 1. Introduction

According to recent estimates, rice (*Oryza sativa* L.) production must be increased by 40% before 2030 to satisfy the demands of the world's growing population [1]. To increase grain production, enhancement of rice yield potential is one of the most important objectives for rice breeding. Since the 1960s, the IRRI has released many high-yielding varieties that have been distributed worldwide [2]. In the late 1980s, a breeding program to develop NPT rice was launched at IRRI to further increase the yield potential of the high-yielding varieties through improvement of plant type. NPT lines had several unique agronomic traits such as large panicles, few unproductive tillers, and large leaves inherited from tropical *japonica* varieties [3,4]. However, despite the improved plant architecture, the NPT lines yielded less than expected, mainly because of low GF [2,4].

Identification and analyses of QTL have improved our understanding of the basis of natural allelic diversity, which is crucial to supporting crop improvement. In the past two decades, many QTL that control traits of agronomic importance have been detected using molecular markers (http://www.gramene.org/). Some of these QTL have been identified by map-based cloning. For example, QTL for TSN have been identified using various segregating populations, including $F_2$ populations, RILs, and double-haploid lines. Several QTL for grain and spikelet number (*QSpp8*, *qSSP1*, *qSSP2*, *qSSP3*, and *qSSP7*, and *gpa7*) have been identified and mapped [5–8], and others (*Gn1a*, *Ghd7*, and *WFP*) have already been cloned [9–11]. However, many of these QTL cannot be used directly in rice-breeding programs because their expressions vary considerably in different genetic backgrounds and environments [12]. Recently, Zhang et al. [13] demonstrated the successful genetic dissection of yield-related traits using ILs with the genetic background of elite rice. ILs carrying small introgressed segments from the donor parents are useful materials for detailed characterization of agronomic traits associated with these specific introgressions.

An *indica* rice variety, IR64 has been accepted as a high-quality and high-yielding variety in many countries [14]. To further improve yield potential and to analyze the genetic basis of yield-related traits, a total of 334 ILs derived from crosses between IR64 and 10 donor varieties (mostly NPT lines) have been developed in our previous study under the IRRI–Japan Collaborative Research Project [15]. Among the 334 ILs, several lines showed agronomically unique characteristics inherited from the NPT lines, such as later heading, longer culm, wider and longer flag leaf, and higher spikelet number than IR64 [16]. By use of the ILs, chromosomal regions associated with agronomic traits such as days to DTH and TSN have been identified. Detailed locations of QTL controlling TSN were identified on chromosome 4 by use of $F_2$ plants derived from crosses between ILs and IR64 [17]. These studies showed that the ILs are useful materials for the genetic analysis of unique agronomic characteristics in high-yielding varieties. Objective of this study was to detect QTL for unique agronomic traits inherited from NPT varieties and valuable in breeding programs. Using marker-assisted selection, NILs carrying these QTL with elite genetic backgrounds were developed and characterized to confirm the effects of the newly detected QTL.

# 2. Materials and methods

## 2.1. Plant materials

In our previous study, $F_1$ plants were generated by crosses between IR64 and 10 donor varieties to develop ILs. These $F_1$ plants were recurrently backcrossed with IR64 three times (Fig. 1). A single $BC_3F_1$ plant was self-pollinated to generate $BC_3F_2$ plants. Similar practice was repeated until the $BC_3F_7$ generation. Agronomic traits in the 334 ILs were almost fixed genetically in $BC_3F_8$ and a genome-wide DNA marker survey revealed that most of the chromosomal regions in the ILs were replaced with the genotype of the recurrent parent, IR64 [15]. In the present study, we selected
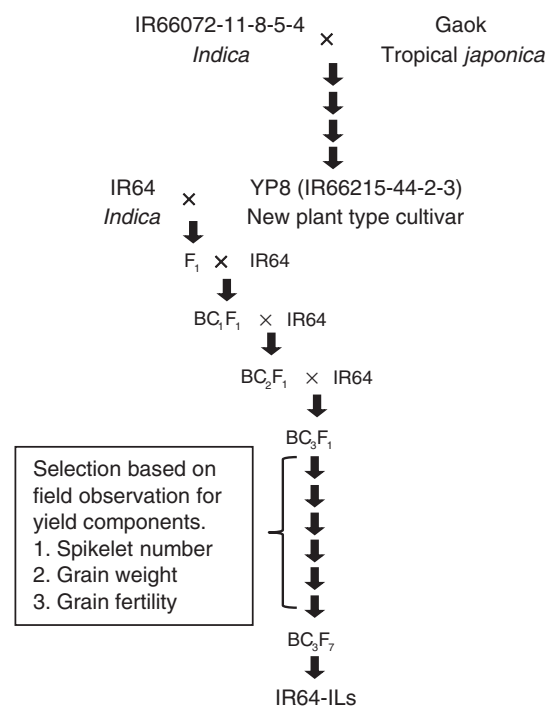


**Fig. 1 – Breeding scheme of development of an introgression line with the genetic background of IR64.**

**Table 1 – Grain yield of IR64 and YTH288 in the experimental field at IRRI (Los Baños, Philippines).**

| Line | Year | Grain yield (g m$^{-2}$) |
|---|---|---|
| YTH288 | 2009 | 654.3 |
| | 2011 | 558.5 |
| IR64 | 2009 | 390.0 |
| | 2011 | 422.5 |

Single plants were planted at 20 cm between rows and 20 cm between hills and grown during the dry season (January–May). Grain was harvested from a 1 × 5 m plot with 2 replications in 2009 and from a 1 × 1 m plot with 3 replications in 2011.

an IL, IR84639-7-28-5-5-2-2-3-2-2-14-B (renamed YTH288), which showed unique agronomic traits such as large panicles and broad leaves inherited from an NPT variety and had higher yield potential than IR64 (Table 1). A NPT variety, IR66215-44-2-3 (renamed YP8), which is a donor parent of YTH288 was derived from a cross between Gaok (a tropical *japonica* variety) and IR66072-11-8-5-4 (IRIS; http://www.iris.irri.org/). One hundred sixty-seven $F_2$ plants were generated from a cross between IR64 and YTH288 and used for QTL mapping for agronomic traits.

From the $F_2$ mapping population, we selected two $F_2$ plants that carried the newly detected QTL on chromosome 2 or 4
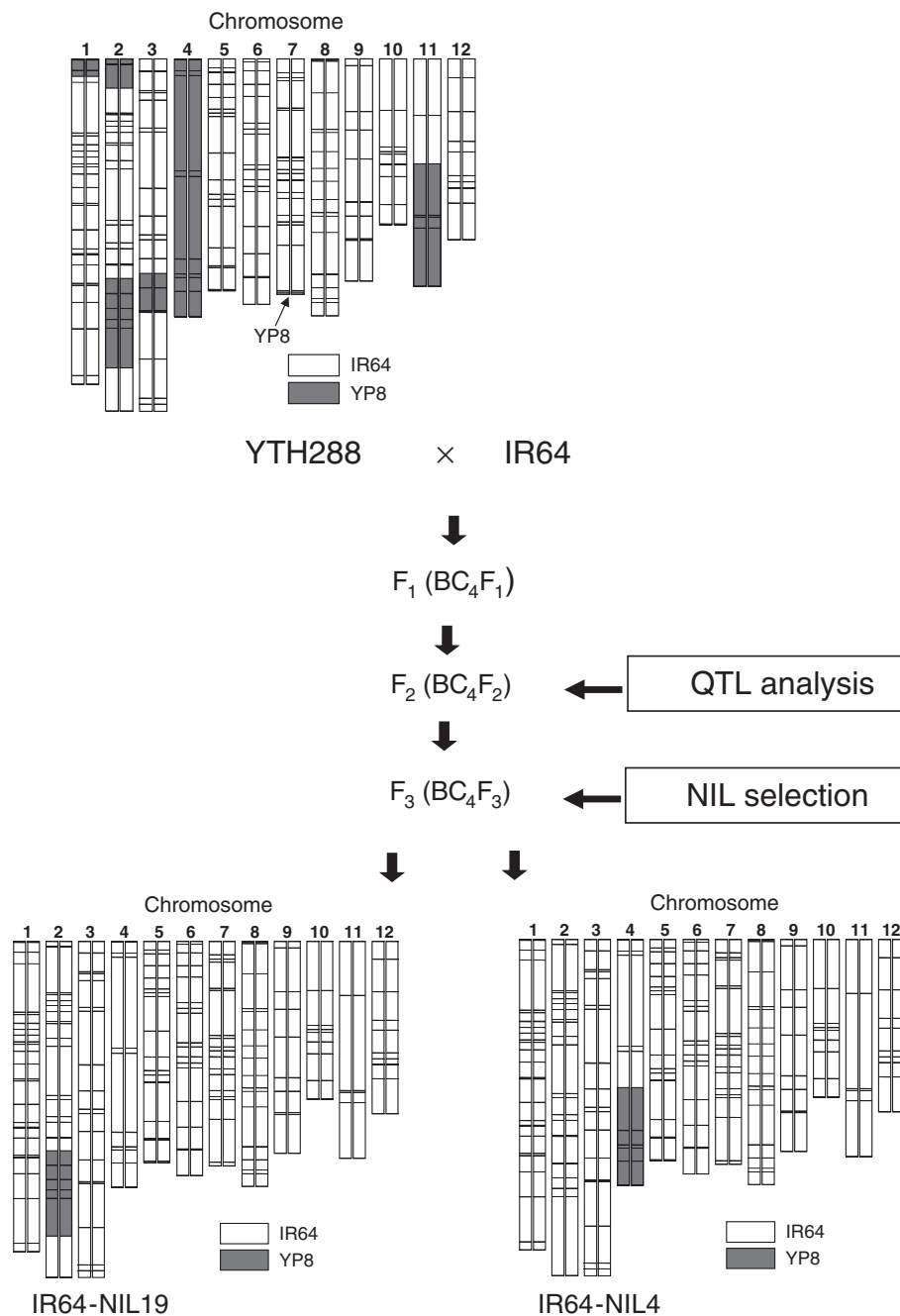


Fig. 2 – Graphical genotypes of YTH288 and of two NILs containing QTL alleles for agronomically important traits: IR64-NIL19 and IR64-NIL4. The 12 pairs of vertical bars represent the chromosomes, and are numbered at the top.

and as few other introgressed segments as possible. $F_3$ lines generated by self-pollination of the selected plants were considered to be NILs. The two NILs were designated IR64-NIL4 (QTL on chromosome 4) and IR64-NIL19 (QTL on chromosome 2) (Fig. 2), and were used for further characterization in the experimental field at IRRI (Los Baños, Laguna, The Philippines; 121°E, 14°N).

In our previous study [17], four NILs of IR64 for QTL for high TSN on chromosome 4 derived from different parents: IR64-NIL2 from IR65564-2-2-3 (renamed YP4), IR64-NIL3 from IR69093-41-2-3-2 (YP5), IR64-NIL5 from IR68522-10-2-2 (YP9), and IR64-NIL6 from IR66750-6-2-1 (YP11) had been developed (Fig. 3). These NILs were used to confirm the effect of QTL on chromosome 4.

### 2.2. Phenotypic evaluation

The $F_2$ plants, IR64, YTH288, and YP8 were grown in an experimental field at IRRI during the dry season (January to May). The approximate day length was 12.5 h. At 21 days after sowing, individual plants were transplanted into the field at a spacing of 20 cm between hills and 30 cm between rows. The $F_2$ plants were individually evaluated for DTH, CL, FLL, FLW, and FSN. DTH was evaluated as the number of days from sowing to flowering. CL was measured from the soil surface to the neck of the tiller that was highest above the ground. FLL and FLW were measured on the flag leaf of the tallest tiller. FSN was evaluated as the number of filled spikelets in a panicle of the tallest tiller. IR64, YTH288, and YP8 were grown together with the $F_2$ population and likewise evaluated using 10 plants per variety. TSN was also evaluated for IR64, YTH288, and YP8 as the sum of the number of filled and unfilled spikelets in the tallest tiller.

To characterize two developed NILs, IR64-NIL4 and IR64-NIL19, CL, FLL, FLW, FSN, and TSN were measured during the 2012 dry season following the same method as used for the $F_2$ plants. IR64-NIL19 was evaluated for DTH in the 2011 dry season.

To confirm the effects of QTL on the long arm of chromosome 4, five NILs and IR64 were evaluated for CL, PL,



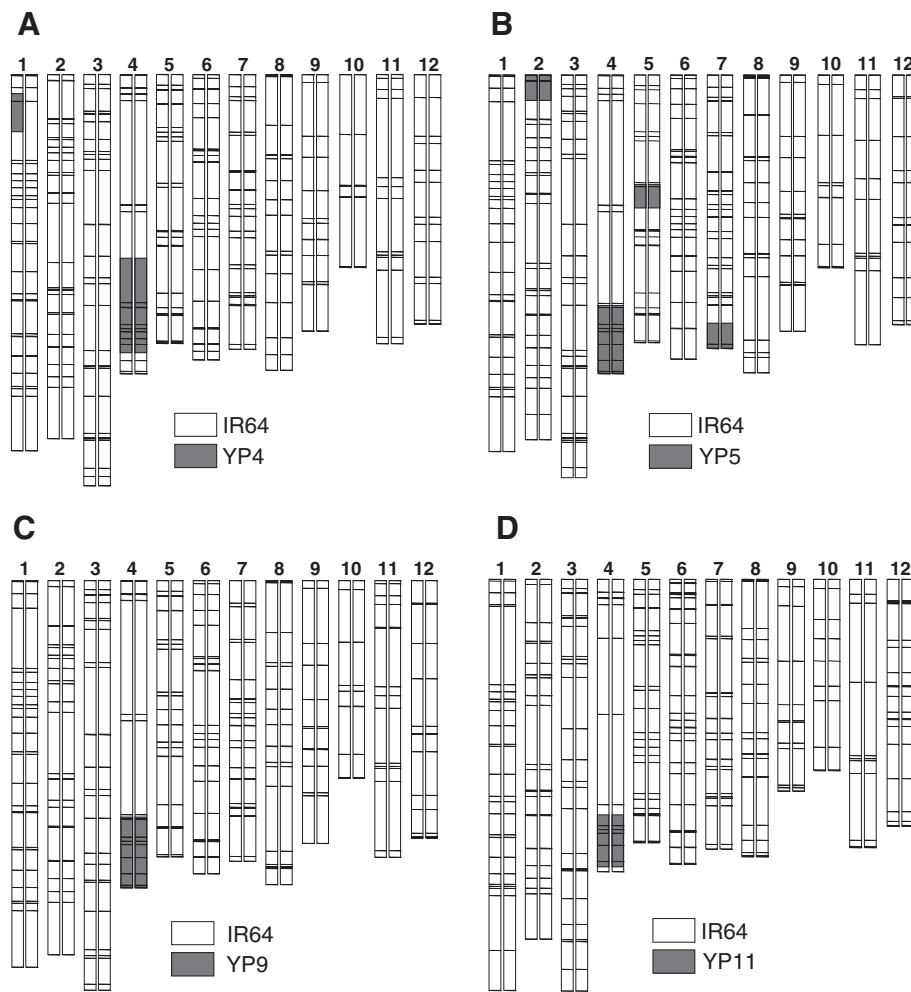Fig. 3 – Graphical genotypes of four NILs with QTL on chromosome 4 derived from NPT varieties. (A) IR64-NIL2; (B) IR64-NIL3; (C) IR64-NIL5; (D) IR64-NIL6. The 12 pairs of vertical bars represent chromosomes, numbered at the top. Horizontal lines across the bars show positions of SSR marker loci. White indicates chromosomal segments from IR64; gray indicates chromosomal segments from the donor parents.

FLL, FLW, and PN in the 2009 dry season. In addition to these traits, LL and LW of the first leaf below the flag leaves were measured for these lines.

### 2.3. Genotyping using SSR markers

Genomic DNA of YTH288, IR64, YP8, and the $F_2$ individuals was extracted from leaf tissue using the CTAB method [18]. A total of 457 SSR markers with known positions distributed across the 12 rice chromosomes were used to survey the polymorphism between IR64 and YP8 [19]. We used 224 of these markers that showed polymorphism between the parents for our genotyping analysis of YTH288. Introgressed segments in YTH288 that derived from YP8 were detected in chromosomes 1, 2, 3, 4, 7, and 11 (see Fig. 2). We used 22 SSR markers that showed the YP8 donor genotype in YTH288 to analyze the $F_2$ individuals: RM3252 and RM3148 on chromosome 1; RM5631, RM5472, and RM240 on chromosome 2; RM2334, RM6329, and RM168 on chromosome 3; RM518, RM1155, RM3843, RM3534, RM17470, RM5503, RM6480, RM6089, RM6365, RM17483, RM17486, and RM6909 on chromosome 4; and RM5349 and RM1341 on chromosome 11. Primer information such as forward and reverse sequences was obtained from http://www.gramene.org/.

We used the PCR to determine SSR marker genotypes. The reactions were performed in a 10-μL PCR mixture containing 1.4 μL of sterile $H_2O$, 1 μL each of 10× PCR buffer containing 20 mmol $L^{-1}$ $Mg^{2+}$ (SBS Genetech, Beijing, China), PCR dye (SBS Genetech), dNTPs (1 mmol $L^{-1}$), 0.1 μL Taq polymerase at 5 U μ$L^{-1}$ (SBS Genetech), 0.25 μL of each forward and reverse SSR primer (5 μmol $L^{-1}$), and 5 μL of DNA (5 ng μ$L^{-1}$) template. PCR amplification was performed in a DNA Engine Dyad thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) with the following profile: an initial denaturation step at 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s; and a final extension step at 72 °C for 5 min and 30 s. PCR products were separated in 4% agarose gels for 1 h at 250 V. Gels were stained with ethidium bromide and visualized under UV light.

### 2.4. QTL analysis

To construct a linkage map around the introgressed segments, we determined the genetic distances between SSR markers for the genotypes in the $F_2$ population. The linkage map was constructed with genetic distances (cM) calculated using the Kosambi map function [20]. The chromosomal locations associated with the QTL for DTH, CL, FLL, FLW, and FSN were determined on the linkage map for the $F_2$ population. Associations between phenotypes and marker genotypes were investigated by simple interval mapping using Windows QTL Cartographer v. 2.5 software [21]. The proportion of the observed phenotypic variation attributable to a given chromosomal region was estimated by using the coefficient of determination ($R^2$). Critical threshold values for the LOD scores for QTL detection were calculated with 1000 permutation tests (with significance at $P < 0.01$).

## 3. Results

### 3.1. QTL analysis for five agronomic traits

Among 334 ILs that had been developed by backcross breeding, YTH288 had several unique agronomic characteristics such as large panicles and broad leaves that were inherited from the NPT rice variety, while the other traits were similar to those of IR64. Compared with IR64, YTH288 had longer DTH and CL, wider FLW, and more FSN (Table 2). YTH288 showed higher FSN and GF than YP8, while TSN of these lines was not significantly different. Continuous distributions were observed for four agronomic traits; CL, FLL, FLW, and FSN in the $F_2$ population derived from the cross between IR64 and YTH288, whereas DTH showed a bimodal distribution (Fig. 4).

QTL analysis using SSR markers on the introgressed segments detected six putative QTL for agronomic traits in YTH288 (Fig. 5, Table 3). Four QTL (for CL, FLL, FLW, and FSN) were detected in the same region on the long arm of chromosome 4, between SSR markers RM6480 and RM3843. These QTL are designated by the letter "q" followed by the trait and then the chromosome number: qCL4.1, qFLL4.1, qFLW4.1, and qFSN4.1. The peak LODs for each QTL fell between RM17470 and RM3534 for qCL4.1, between RM6909 and RM3843 for qFLL4.1, between RM3534 and RM17483 for qFLW4.1, and between RM6480 and RM5503 for qFSN4.1. The other two QTL, designated as qDTH2.1 and qFLL2.1, were both located between RM5472 and RM240 on the long arm of chromosome 2. Each of the QTL explained 9% to 28% of the phenotypic variance. All QTL with the YP8 allele, except for qFLL2.1, increased the phenotypic value of the trait they controlled.

| Table 2 – Characterization of agronomic traits of IR64 and YTH288 in the dry season in 2009. | | | | | | | |
|---|---|---|---|---|---|---|---|
| Line | Agronomic trait (mean ± SD) [a] | | | | | | |
| | DTH [b] | CL (cm) | FLL (cm) | FLW (cm) | FSN | TSN | GF (%) |
| IR64 | 85.0 ± 1.0 | 70.1 ± 3.2 A | 38.2 ± 4.3 A | 1.3 ± 0.1 A | 101.3 ± 14.0 A | 138.0 ± 21.8 A | 74.0 ± 6.3 B |
| YP8 | – | 83.6 ± 4.2 B | 41.5 ± 2.4 A | 1.8 ± 0.1 C | 112.7 ± 20.4 A | 197.7 ± 34.1 B | 57.7 ± 11.1 A |
| YTH288 | 93.1 ± 2.8 | 81.4 ± 2.9 B | 38.1 ± 4.0 A | 1.5 ± 0.1 B | 164.1 ± 23.8 B | 201.6 ± 22.8 B | 81.7 ± 6.7 B |

Different letters indicate significant differences ($P < 0.01$, Tukey–Kramer test).
[a] DTH, days to heading; CL, culm length (cm); FLL, flag leaf length (cm); FLW, flag leaf width (cm); FSN, filled spikelet number per panicle; TSN, total spikelet number per panicle; and GF, grain fertility.
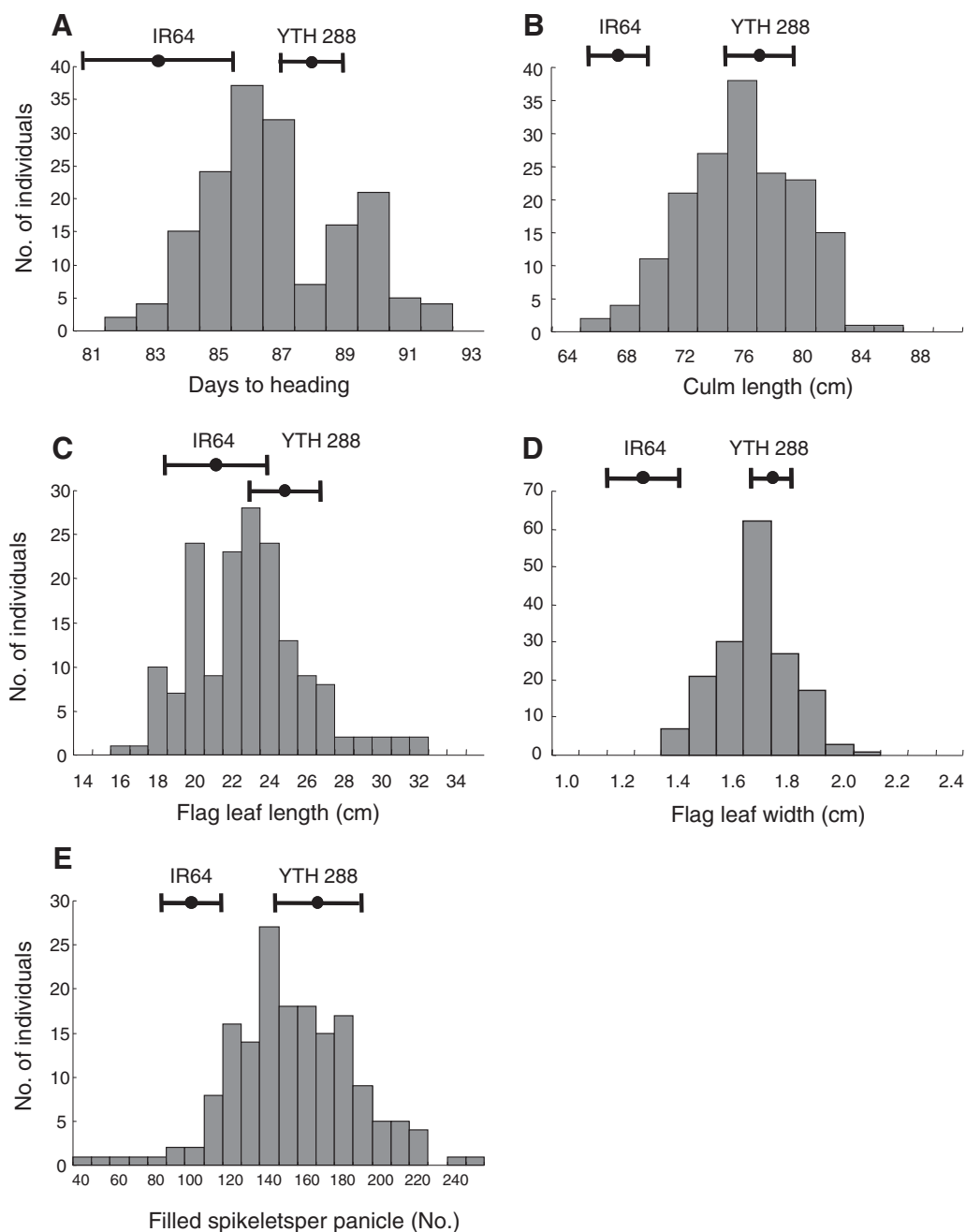[b] Calculated from data obtained in three years.

Fig. 4 – Frequency distributions of five agronomic traits in the F₂ population derived from a cross between IR64 and YTH288 (N = 167). (A) days to heading; (B) culm length; (C) flag leaf length; (D) flag leaf width; and (E) filled spikelet number per panicle. The dots and error bars at the top of each graph represent the mean and standard deviation of the agronomic trait of each parent.

### 3.2. Characterization of NILs for the detected QTL

To confirm the effects of QTL for these agronomic traits, we developed two NILs with the IR64 genetic background that carried the detected QTL on either chromosome 2 or chromosome 4 (Fig. 2). These two NILs, designated as IR64-NIL4 and IR64-NIL19, were evaluated for DTH, CL, FLL, FLW, FSN, and TSN. IR64-NIL19, which carries *qDTH2.1* and *qFLL2.1* on chromosome 2, flowered two days later than IR64 but showed no significant difference for other traits (Table 4). IR64-NIL4,

which carries *qCL4.1*, *qFLL4.1*, *qFLW4.1*, and *qFSN4.1* on chromosome 4, showed significantly higher CL, FLL, FLW, and TSN than IR64.

### 3.3. Characterization of NILs carrying QTL on chromosome 4 derived from different parents

To evaluate effect of the QTL on agronomic traits, five NILs carrying introgressed segments on chromosome 4 derived from different donor parents were compared with IR64. These
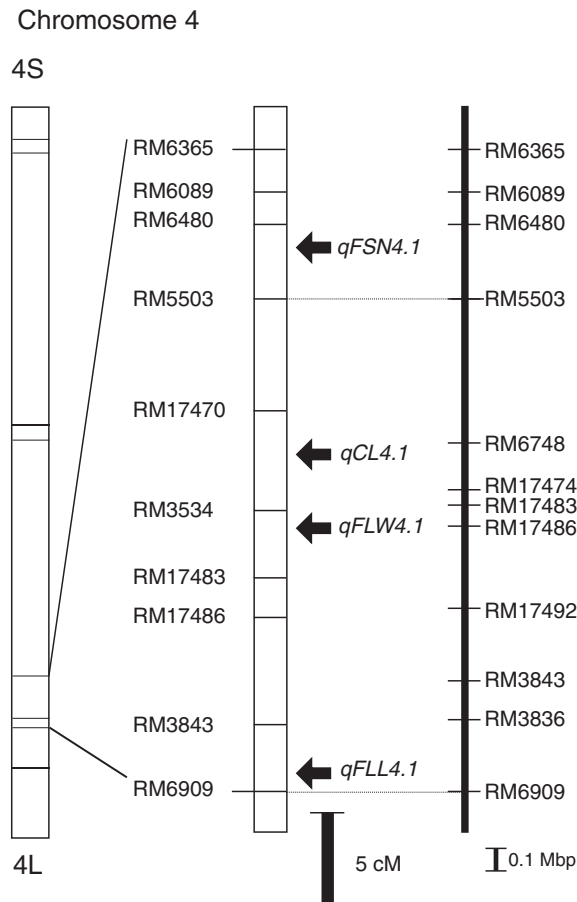
**Fig. 5 – Mapping of the QTL for agronomic traits on chromosome 4. Positions of the simple sequence repeat markers on chromosome 4 were detected. The arrows indicate the location of four putative QTL: FSN (filled spikelet number per panicle), CL (culm length), FLW (flag leaf width), and FLL (flag leaf length).**

NILs showed significant differences from IR64 for several agronomic traits. All five NILs showed significantly higher CL, FLL, LW, and FLW than IR64 (Table 5). These NILs also showed greater PL than IR64, whereas the difference between IR64 and IR64-NIL2 was not significant. LL of these NILs was also greater than that of IR64, and significantly so in IR64-NIL2,

IR64-NIL5, and IR64-NIL6. PN values of IR64-NIL2, IR64-NIL3 and IR64-NIL5 were significantly lower than that of IR64, whereas those of IR64-NIL5 and IR64-NIL6 were not significantly different from that of IR64. These five NILs showed significantly greater FSN than IR64 in our previous study (see Table 3 in [17]).

## 4. Discussion

### 4.1. Newly detected QTL for agronomic traits

QTL for agronomic traits in rice have been identified in all chromosomal regions. Yonemaru et al. [22] reported co-localization of QTL and distribution of QTL clusters associated with multiple morphological traits on chromosomes 1, 3, 4, 6, and 9. Among the QTL clusters, many QTL for morphological traits such as leaf size, spikelet number, PN, and root volume were located on the long arm of chromosome 4 (http://qtaro.abr.affrc.go.jp/). In the present study, four QTL for CL, FLL, FLW, and FSN were colocalized on the long arm of chromosome 4 (Fig. 5). In previous studies, QTL for various agronomic traits were identified in the same region of chromosome 4: GW and plant height [23]; FLL and FLW [24]; FLL, FLW, FLA, PL, PN, and grain yield [25,26]; FLW and PL [27]; and FLW and root volume per tiller [28]. This colocalization of QTL for various traits may represent a gene cluster or a pleiotropic effect of a gene underlying the QTL. Precise analysis will be needed to identify the detailed locations of the QTL derived from NPT varieties on the long arm of chromosome 4.

We detected QTL for unique agronomic traits such as large panicles and broad leaves that may contribute to higher yield of YTH288 than IR64. Among them were QTL associated with leaf size, qFLL4.1 and qFLW4.1, on the long arm of chromosome 4 where Yue et al. [26] detected QFll4, QFlw4, and QFla4. We identified qFLL4.1 between RM3843 (32.2 Mb) and RM6909 (32.6 Mb) and qFLW4.1 between RM3534 (31.7 Mb) and RM17483 (31.7 Mb). In contrast, QFll4, QFlw4, and QFla4 were detected in the interval between RM255 (31.5 Mb) and RM349 (33.2 Mb). Besides leaf size, we detected a QTL associated with panicle structure, qFSN4.1 on the long arm of chromosome 4 in the interval between RM6480 (30.4 Mb) and RM5503 (30.9 Mb), whereas qNOS4-3 was identified around RG214 (32.3 Mb) and qSN-4a was detected in the interval between RM317 (29.7 Mb) and RM255 (31.5 Mb) [25,29]. Furthermore, a

**Table 3 – QTL associated with five agronomic traits in the F₂ population derived from a cross between IR64 and YTH288 using simple interval mapping.**

| Trait[a] | QTL | Marker interval | Chromosome | LOD score | $R^{2}$[b] | Additive effect[c] | Dominant effect[c] |
|---|---|---|---|---|---|---|---|
| DTH | qDTH2.1 | RM5472–RM240 | 2 | 4.6 | 0.12 | −1.1 | 0.2 |
| CL | qCL4.1 | RM17470–RM3534 | 4 | 4.6 | 0.13 | −0.8 | 2.3 |
| FLL | qFLL2.1 | RM5472–RM240 | 2 | 3.7 | 0.10 | 1.3 | 0.5 |
| FLL | qFLL4.1 | RM6909–RM3843 | 4 | 6.0 | 0.15 | −1.6 | −0.7 |
| FLW | qFLW4.1 | RM3534–RM17483 | 4 | 12.2 | 0.28 | −0.1 | 0.0 |
| FSN | qFSN4.1 | RM6480–RM5503 | 4 | 3.1 | 0.09 | −14.6 | −0.1 |

[a] DTH, days to heading; CL, culm length; FLL, flag leaf length; FLW, flag leaf width; and FSN, filled spikelet number per panicle.
[b] Proportion of the phenotypic variation explained by each QTL.
[c] The values indicate the relative effect of the IR64 alleles.

**Table 4 – Agronomic characteristics of IR64 and developed NILs.**

| Line | Introgressed segment on | Agronomic trait [a] (mean ± SD) | | | | | |
|------|-------------------------|------|---------|----------|----------|--------|--------|
|      |                         | DTH | CL (cm) | FLL (cm) | FLW (cm) | FSN | TSN |
| IR64 | – | 86 ± 1.8 | 65.2 ± 1.9 | 26.6 ± 3.9 | 1.3 ± 0.2 | 96.0 ± 18.8 | 105.7 ± 19.6 |
| IR64-NIL19 | Chr. 2 | 88 ± 1.6[*] | 68.8 ± 3.8 | 23.3 ± 2.4 | 1.3 ± 0.1 | 104.3 ± 13.3 | 118.3 ± 11.3 |
| IR64-NIL4 | Chr. 4 | NA | 75.6 ± 5.2[*] | 32.4 ± 4.1[*] | 1.9 ± 0.1[*] | 116.6 ± 30.9 | 197.8 ± 27.5[*] |

[a] DTH, days to heading; CL, culm length; FLL, flag leaf length; FLW, flag leaf width; FSN, filled spikelet number per panicle; and TSN, total spikelet number per panicle. The agronomic traits were calculated from data obtained in the 2012 dry season except for DTH, obtained in the 2011 dry season. Values represent means and standard deviations with significance levels (by t-test). *P < 0.05.

QTL for FLW, spikelet number and root volume has been mapped within 38 Kb on the long arm of chromosome 4 [28]. Recently, a gene responsible for increase in TSN on this chromosomal region was identified by Fujita et al. [30]. To determine whether the detected QTL are the same as those previously reported, more detailed analysis is needed.

We also detected QTL for agronomic traits qDTH2.1 and qFLL2.1 on the long arm of chromosome 2 where QTL for similar traits were previously identified. The location of qDTH2.1 is similar to that where Hd7 was reported [31]. C560, the nearest RFLP marker for Hd7, was identified on the long arm of chromosome 2 and Hd7 was located between R3393 (29.2 Mb) and R2511 (35.9 Mb), while qDTH2.1 was detected around RM5472 (31.5 Mb) to RM240 (32.4 Mb). The location of Hd7 overlapped with that of qDTH2.1.

### 4.2. Effects of detected QTL on chromosomes 4 from NPT rice varieties

In 1990s, NPT rice varieties had been developed using tropical japonica varieties to enhance the yield potential of rice. However, the grain yield of NPT variety was not higher than expected, mainly because of low GF [4]. To eliminate genetic factors associated with low GF, the NPT variety YP8 was recurrently backcrossed to IR64 and ILs with unique agronomic traits inherited from NPT varieties were developed [15]. One of the ILs, YTH288, showed unique agronomic traits such as heavy panicles and broad leaves inherited from a NPT variety, YP8, but GF of YTH288 was higher than that of YP8. In the $F_2$ population of a cross between YTH288

and IR64, QTL for several agronomic traits were detected on the same location on the long arm of chromosome 4. Effects on multiple traits were commonly observed among NILs for chromosome 4 derived from different NPT parents: YP4, YP5, YP8, YP9, and YP11, in addition to increased TSN as observed previously by Fujita et al. [17]. These results suggested that a QTL cluster or pleiotropic QTL is a major genetic factor influencing the unique agronomic traits of NPT varieties.

IR64-NIL4, which carries an introgressed segment containing qCL4.1, qFLL4.1, qFLW4.1, and qFSN4.1 on the long arm of chromosome 4, showed greater CL, FLL, FLW, and FSN than IR64 (Table 3). Although the difference in FSN between IR64 and IR64-NIL4 was not significant, the TSN of the NIL was significantly greater than that of IR64. These NILs for qTSN4 are promising lines for enhancing yield potential with fewer undesirable traits related to grain yield in rice.

## 5. Conclusion

Using IL of IR64, we successfully detected QTL for unique agronomic traits in a NPT variety. Effects of the QTL were confirmed by development of two NILs that each carried an introgressed segment from YP8 on chromosome 2 or 4 in the IR64 genetic background. By using developed NILs, only valuable agronomic traits can be isolated from NPT varieties for rice breeding programs. These NILs could thus be used as breeding materials to improve yield potential in rice and as research materials for studying agronomic traits.

**Table 5 – Agronomic trait data of near-isogenic lines with introgressed segments on chromosome 4 in the dry season of 2009.**

| Line | Donor [a] | Agronomic trait (mean ± SD) [b] | | | | | | |
|------|-----------|---------|---------|----------|----------|----------|----------|------|
|      |           | CL (cm) | PL (cm) | LL (cm) | FLL (cm) | LW (cm) | FLW (cm) | PN |
| IR64 |  | 70.5 ± 2.2 | 25.6 ± 1.3 | 38.4 ± 3.0 | 25.9 ± 1.4 | 1.3 ± 0.1 | 1.5 ± 0.1 | 18.9 ± 3.4 |
| IR64-NIL2 | YP4 | 81.1 ± 2.5* | 26.6 ± 1.7 | 48.3 ± 8.4* | 32.7 ± 4.4* | 1.7 ± 0.1* | 2.0 ± 0.1* | 14.2 ± 2.4* |
| IR64-NIL3 | YP5 | 83.6 ± 2.8* | 27.4 ± 1.3* | 45.0 ± 7.0 | 30.3 ± 4.1* | 1.6 ± 0.1* | 1.9 ± 0.1* | 14.9 ± 3.2* |
| IR64-NIL4 | YP8 | 78.2 ± 1.4* | 27.7 ± 1.6* | 43.0 ± 4.3 | 33.1 ± 2.7* | 1.6 ± 0.1* | 2.0 ± 0.1* | 13.9 ± 2.9* |
| IR64-NIL5 | YP9 | 77.5 ± 3.1* | 27.8 ± 1.2* | 50.2 ± 5.3* | 33.0 ± 3.6* | 1.6 ± 0.1* | 1.9 ± 0.1* | 20.3 ± 4.8 |
| IR64-NIL6 | YP11 | 75.1 ± 2.4* | 28.4 ± 0.8* | 49.8 ± 5.6* | 33.5 ± 3.7* | 1.5 ± 0.1* | 1.8 ± 0.1* | 18.2 ± 2.2 |

[a] YP4, IR65564-2-2-3; YP5, IR69093-41-2-3-2; YP8, IR66215-44-2-3; YP9, IR68522-10-2-2; and YP11, IR66750-6-2-1.
[b] More than eight plants in each line were investigated and the means and standard deviations are shown. Means accompanied by asterisks are significantly different between IR64 and NILs at the 5% level according to Dunnett's test. CL, culm length; PL, panicle length; LL, leaf length; FLL, flag leaf length; LW, leaf width; FLW, flag leaf width; and PN, panicle number per plant.

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