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REVIEW

Genetic Dissection of Agronomic Traits in Introgression Lines and Improvement of an Elite *Indica* Rice Variety

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Abstract

To enhance the yield potential of rice by improving plant type, we have developed introgression lines (ILs: BC_3-derived lines) with the genetic background of the elite *indica* variety IR64. A total of 334 ILs derived from crosses between IR64 as the recurrent parent and 10 donor parents (nine new-plant-type lines IR65600-87-2-2-3, IR65598-112-2, IR65564-2-2-3, IR69093-41-2-3-2, IR69125-25-3-1-1, IR66215-44-2-3, IR68522-10-2-2, IR71195-AC1 and IR66750-6-2-1, and one Japanese high-yielding cultivar, 'Hoshiaoba') have been developed by recurrent backcross breeding. The agronomic traits of the 334 ILs were evaluated in the experimental field of the International Rice Research Institute from 2005 to 2007, and their genotypes were determined using SSR markers. Several agronomic traits (days to heading, leaf length and width, culm and panicle length, number of panicles, total spikelet number per panicle, and 100-grain weight) were dissected genetically by QTL analysis using hybrid populations derived from crosses between IR64 and ILs with unique traits. More than 30 QTLs were detected and their effects were confirmed by developing and evaluating near-isogenic lines (NILs). These ILs and NILs are useful for both breeding and further genetic dissection of agronomic traits across various environments.

Discipline: Plant breeding
Additional key words: grain yield, near-isogenic line, new plant type, *Oryza sativa*, quantitative trait locus

Introduction

Rice (*Oryza sativa* L.) is an essential cereal worldwide; it is consumed as a staple food by more than three billion people, mainly in Asia. Enhancement of the yield potential of rice is one of the most important breeding objectives. Since the 1960s, high-yielding rice varieties such as IR64 bred by the International Rice Research Institute (IRRI) have been distributed worldwide and used by plant breeders and farmers. In the late 1980s, IRRI launched a breeding program to develop new-plant-type (NPT) rice, in order to increase the yield potential of inbred rice varieties in the tropics. The NPT varieties exhibited several unique traits derived from tropical *japonica*: low-tillering habit, few unproductive tillers, large panicles, thick culm, lodging resistance, and large dark green flag leaves (Khush 1995). However, despite their favorable agronomic traits, the NPT varieties yield less than modern *indica* cultivars, mainly due to low grain fertility and low panicle number (Peng et al. 1999, Peng & Khush 2003).

The genetic dissection of agronomic traits is crucial for crop improvement. In the past two decades, many quantitative trait loci (QTLs) that control the traits of agronomic importance have been detected, and some were cloned by using molecular markers (www.gramene.org). The grain yield of rice is determined by four components: total spikelet number per panicle (TSN), panicle number per plant (PN), grain weight, and spikelet fertility. QTLs for

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TSN have been identified using F_2 populations, recombinant inbred lines, and double-haploid lines. Several QTLs for grain and spikelet number have been identified and mapped (*QSpp8*, *qSSP1*, *qSSP2*, *qSSP3*, *qSSP7*, and *gpa7*, Tian et al. 2006, Zhang et al. 2006, 2009, Xing et al. 2008) or cloned (*Gn1a*, *DEP1*, *OsSPL14*, *APO1*/*SCM2*, and *Ghd7*, Ashikari et al. 2005, Xue et al. 2008, Huang et al. 2009, Ikeda-Kawakatsu et al. 2009, Jiao et al. 2010, Miura et al. 2010, Ookawa et al. 2010). Seven QTLs directly affecting the grain weight in rice have been cloned: *GS3* (Fan et al. 2006), *GW2* (Song et al. 2007), *qSW5*/*GW5* (Shomura et al. 2008, Weng et al. 2008), *GS5* (Li et al. 2011), *GL3.1*/*qGL3* (Qi et al. 2012, Zhang et al. 2012), *GW8* (Wang et al. 2012), and *TGW6* (Ishimaru et al. 2013). However, many of these QTLs cannot be used directly in rice-breeding programs because their expression varies considerably in different genetic backgrounds and environments (Li et al. 2003, Yu et al. 2002). Recently, Zhang et al. (2013) genetically dissected yield-related traits using introgression lines (ILs) with the genetic background of elite rice.

IR64 was released in 1985 and has since been widely accepted as a high-quality rice variety in many countries (Khush 1987). Given this wide adaptability, breeding materials with the IR64 genetic background, such as double-haploid lines, recombinant inbred lines, and mutant lines, have been developed for researching and improving rice varieties (Guiderdoni et al. 1992, Wu et al. 2005).

Under the IRRI-Japan Collaborative Research Project, we tried to enhance the yield potential of IR64 by using NPT varieties that have larger flag leaves, a higher spikelet number, and heavier grains than IR64. A total of 334 ILs were developed with the genetic background of IR64 by backcrossing nine NPT varieties and one Japanese high-yielding cultivar as donor parents with IR64 as the recurrent parent (Fujita et al. 2009). The ILs with favorable yield-related traits and few undesirable agronomic traits were selected in the BC_3 generation by field observation (Fujita et al. 2009). Using the developed ILs, we have genetically dissected the valuable traits of NPT varieties.

In this paper, we review a series of our rice breeding studies: (1) development and characterization of ILs for unique NPT agronomic traits with the genetic background of IR64; (2) detection of QTLs for these traits by using hybrid populations derived from crosses between IR64 and its ILs; and (3) development and characterization of NILs for the detected QTLs.

Development and characterization of introgression lines with the genetic background of IR64

1. Development of ILs

Figure 1 shows the breeding scheme used to develop ILs with the genetic background of IR64. IR65600-87-2-2-3 (designated as YP1), IR65598-112-2 (YP3), IR65564-2-2-3 (YP4), IR69093-41-2-3-2 (YP5), IR69125-25-3-1-1 (YP6), Hoshiaoba (YP7), IR66215-44-2-3 (YP8), IR68522-10-2-2 (YP9), IR71195-AC1 (YP10), and IR66750-6-2-1 (YP11) were used as donor parents (Table 1). The nine varieties with IR numbers were NPT varieties with broad leaves, thick stems, large root systems, and few large panicles (Peng et al. 1999). Hoshiaoba (also known as 'Chugoku 146') was a high-yielding cultivar developed in Japan using *indica* and *japonica* varieties (Maeda et al. 2003).

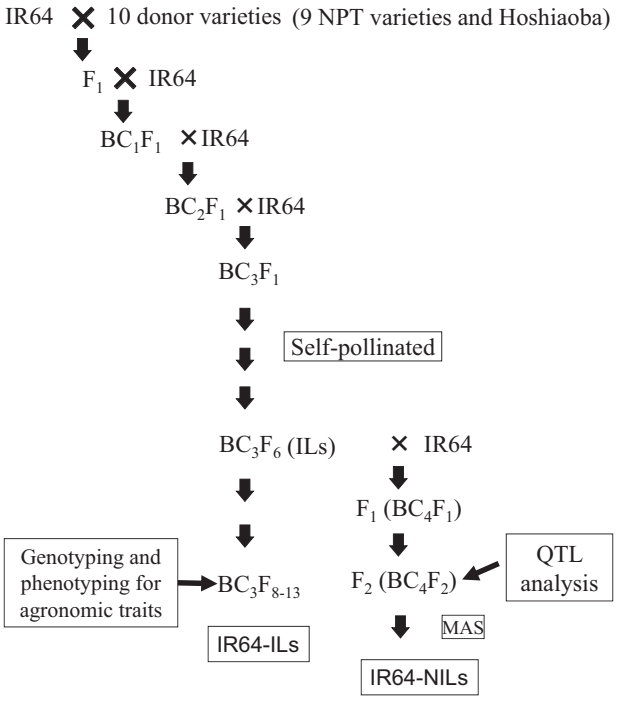

Fig. 1. Breeding scheme for the development of introgression lines (ILs) derived from 10 donor parents with the IR64 genetic background and their segregating populations for QTL analysis and selection of near isogenic lines
MAS: marker-assisted selection; NIL: near-isogenic lines; NPT: new-plant-type.

F_1 plants generated by crossing IR64 and the 10 donor varieties were backcrossed with IR64 three times. A single BC_3F_1 plant for each donor parent was selected on the basis of its agronomic traits (i.e., heading date, tiller number, plant height, panicle length [PL], 100-grain weight [GW]) and continuously self-pollinated. Their progeny that had favorable yield-related traits and few undesirable agronomic traits were selected through field observation. The agronomic traits in ILs were considered to be genetically fixed in BC_3F_8 and then evaluated in the following generations. A total of 334 ILs were developed, which included 10 sib IL groups ranging from 16 lines for YP9 to 56 lines for YP5 (Table 1).

Table 1. Introgression lines developed from crosses between IR64 and 10 donor parents (Fujita et al. 2010)

Entry No.	Donor	Varieties in pedigree	Developed introgression lines			
			No. of lines	Designation	from	to
YP1	IR65600-87-2-2-3	Shen Nung 89-366, Ketan Lumbu	36	YTH	2 –	38
YP3	IR65598-112-2	Shen Nung 89-366, Genjah Wangkal	23	YTH	55 –	78
YP4	IR65564-2-2-3	NO 11, Bali Ontjer	45	YTH	79 –	126
YP5	IR69093-41-2-3-2	Shen Nung 89-366, Ketan Lumbu, Gundil Kuning	56	YTH	169 –	227
YP6	IR69125-25-3-1-1	Shen Nung 89-366, Ketan Lumbu, Gundil Kuning	29	YTH	229 –	258
YP7	Hoshiaoba	Chugoku 113, Oochikara	21	YTH	259 –	280
YP8	IR66215-44-2-3	Gaok, Chir 87-3-1, Moroberekan, Palawan	29	YTH	282 –	311
YP9	IR68522-10-2-2	Moroberekan, Shen Nung 89-366, Daringan	16	YTH	312 –	328
YP10	IR71195-AC1	Shen Nung 89-366, Pring, Akihikari, Cnax 1419-37-2-3-4, Mee Nteri	39	YTH	329 –	369
YP11	IR66750-6-2-1	Shen Nung 89-366, Sri Kuning	40	YTH	127 –	168

Donors were developed from crosses among several vareties including *japonica* varieties.

2. Genotyping ILs using SSR markers

Total genomic DNA of each of the 334 ILs (BC_3F_8 plants) was analyzed using simple sequence repeat (SSR) markers. A total of 457 SSR markers (McCouch et al. 2002) of known chromosomal position were used to survey the polymorphism between IR64 and the donor parents. More than 200 of these markers (ranging from 224 in YP8 to 280 in YP10) were polymorphic between the parents and used for IL genotyping (Fujita et al. 2010a). These markers were distributed across 12 chromosomes at an average genetic distance between markers of approximately 6.4 cM.

Fujita et al. (2009, 2010a, b) described the results of IL genotyping. On chromosome 1 (120-130 cM from the end of the short arm), introgressed segments from the YP4, YP5, and YP11 donor parents were found in 67 ILs. On chromosome 2 (0-10 cM), introgressed segments from YP1, YP5, YP6, YP8, YP9, and YP10 were found in 127 ILs. On chromosome 4 (100-110 cM), introgressed segments from YP1, YP4, YP5, YP8, YP9, and YP11 were found in 107 ILs. On chromosome 5 (40-50 cM), introgressed segments from YP1, YP3, YP5, YP9, YP10, and YP11 were found in 97 ILs. On chromosome 6 (70-80 cM), introgressed segments from YP5, YP6, YP7, and YP10 were found in 67 ILs. These segments of chromosomes 1, 2, 4, 5, and 6 were often found in four sib IL groups.

3. Phenotypic evaluation of agronomic traits of ILs

All ILs were grown in the experimental field at IRRI, Los Baños, Laguna, Philippines, during the dry season (DS: January to May) and the wet season (WS: July to November) from 2005 to 2007. Each line was represented by at least two rows of 12 plants per row. At 21 days after sowing, plants were transplanted (20 cm between hills and 30 cm between rows). Days to heading (DTH), culm length (CL), PL, leaf width (LW), leaf length (LL), and PN were evaluated in 2005WS, 2006DS, 2006WS, and 2007DS. TSN and GW were only evaluated in 2007DS.

DTH was evaluated as the number of days from sowing until 50% of the panicles flowered. For CL, LW, LL, PL, PN, GW, and TSN, 10 individuals from the middle of each row were assessed, and an average value for each trait was recorded. CL was measured from the soil surface to the neck of the tallest tiller in a plant. PL was measured from the panicle neck to the panicle tip of the tallest tiller. LW and LL were measured on the second leaf under the flag leaf of the main tiller. PN was determined by counting the productive panicles per plant. GW was measured by weighing 100 filled grains. TSN was the sum of filled and unfilled spikelets per panicle.

The donor parents had lower PN and higher LW, GW, and TSN than did IR64. Several ILs showed characteristics similar to those of the donor parents, although variation in the agronomic characteristics in each sib IL group was different. In fact, wide variation was observed (see Table 4.1 in Fujita et al. 2010a).

4. Association of chromosome regions with agronomic traits

Based on the phenotypes and genotypes of the 334 ILs, associations between agronomic traits and introgressed chromosomal segments were investigated. A total of 54 regions were associated with agronomic traits (7 for DTH, 8 for CL, 8 for LW, 4 for LL, 6 for PL, 3 for PN, 7 for GW, and 11 for TSN; Fig. 2).

Several ILs derived from each donor parent had lower PN, higher LW, and higher GW and/or TSN than IR64. The association of several introgressed chromosome regions with these agronomic traits which are related to grain yield

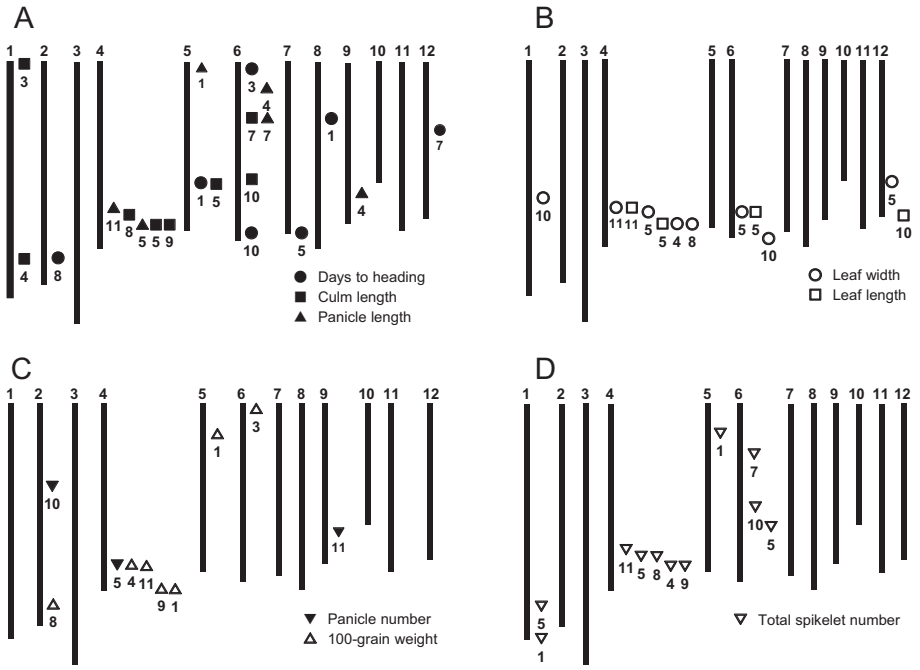

Fig. 2. QTLs for eight agronomic traits in 334 IR64 ILs (Fujita et al. 2009)
Positions of QTLs for A: days to heading, culm length, and panicle length; B: leaf length and width; C: panicle number and 100-grain weight; D: total spikelet number per panicle. The numbers below symbols are YP numbers (donor parent numbers).

was revealed using SSR markers. Previous studies showed that the leaf size (LW, LL, and leaf area) is correlated with grain yield (Cui et al. 2003, Yue et al. 2006). Several QTLs for leaf area and grain yield were detected in the same region. The ILs with low PN and high LW, GW, and TSN are useful for further genetic analysis.

5. Further characterization of ILs in agronomic studies

In addition to genetic analysis, several ILs were used for agronomic studies. The responses of 21 ILs were evaluated under drought-prone rainfed lowland conditions versus continuous flooding (Kano-Nakata et al. 2013). Among the 21 ILs, YTH183 and YTH304 were used to clarify the functional role of plasticity in root system development for biomass production and water uptake under drought-prone conditions. Kato et al. (2011) further elucidated the physiological characteristics behind the high performance of YTH183, which is adapted to water-saving cultivation.

Bueno et al. (2010) investigated water productivity and morphological traits for adaptation to the water-saving cultivation of various lines including YTH183 and YTH304, which are adapted to aerobic conditions. YTH183 was found to perform well under continuous flooding and alternatively wetting/drying conditions, and highly efficient in biomass partitioning. YTH304 was assumed to perform well under continuous flooding and alternatively wetting/drying conditions, but with low efficiency in biomass partitioning.

Okami et al. (2014) evaluated the agronomic performance of YTH323, which has fewer tillers and larger leaves than IR64, under aerobic conditions. It was revealed that genetic modification of the aboveground architecture of IR64, a typical tropical lowland rice cultivar, to reduce tiller and leaf number improves adaptation to aerobic culture. Furthermore, to investigate the traits and mechanisms associated with the aboveground morphological response to re-watering after an early-season drought, Okami et al. (2015) compared IR64, YTH323, and its donor parent YP9, which have distinct plant architectures, and found that the rapid increase in tiller number upon re-watering in IR64 was associated with the low amount of assimilate required to produce new tillers, instead of a short phyllochron.

Detection of QTLs for unique agronomic traits in F_2 populations derived from crosses between IR64 and its ILs

1. Plant materials for QTL analysis

To genetically map QTLs for unique agronomic traits, a total of 16 ILs among the 334 ILs were used. These lines were selected for their high TSN and GW, low or high DTH, and large leaf size compared with IR64, and then crossed with IR64 to generate F_2 (equivalent to BC_4F_2) populations for QTL analysis (Table 2).

Table 2. QTLs for unique agronomic traits detected by using segregating populations derived from crosses between IR64 ILs and IR64

Trait (Abbreviation)	QTL[a]	Chromosome	Donor IL	Developed NIL[a]	Reference	QTL name in reference
Days to heading (DTH)						
	qDTH8.1 -YP1	8	YTH 12	IR64-NIL7	Fujita et al. 2011	*qDTH8*[yp1]
	qDTH6.1 -YP3	6	YTH 74	IR64-NIL8	Fujita et al. 2011	*qDTH6*[yp3]
	qDTH11.1 -YP6	11	YTH 252	IR64-NIL9	Fujita et al. 2011	*qDTH11*[yp6]
	qDTH6.2 -YP7	6	YTH 277	IR64-NIL10	Fujita et al. 2011	*qDTH6*[yp7]
	qDTH11.2 -YP7	11	YTH 277	IR64-NIL11	Fujita et al. 2011	*qDTH11*[yp7]
	qDTH4.1 -YP5	4	YTH 222	IR64-NIL17	unpublished	
	qDTH8.2 -YP5	8	YTH 222	IR64-NIL18	unpublished	
	qDTH2.1 -YP8	2	YTH 288	IR64-NIL19	Tagle et al. 2016	*qDTH2.1*
	qDTH6.3 -YP10	6	YTH 339	IR64-NIL20	unpublished	
Total spikelet number (TSN)						
	qTSN4.1 -YP4	4	YTH 83	IR64-NIL2	Fujita et al. 2012	*qTSN4*[YP4]
	qTSN4.2 -YP5	4	YTH 191	IR64-NIL3	Fujita et al. 2012	*qTSN4*[YP5]
	qTSN4.3 -YP8	4	YTH 288	IR64-NIL4	Fujita et al. 2012	*qTSN4*[YP8]
	qTSN4.4 -YP9	4	YTH 326	IR64-NIL5	Fujita et al. 2012	*qTSN4*[YP9]
	qTSN4.5 -YP11	4	YTH 155	IR64-NIL6	Fujita et al. 2012	*qTSN4*[YP11]
	qTSN7.1 -YP7	7	YTH 270	IR64-NIL13	Koide et al. 2013	*qTSN7.1*
	qTSN12.1 -YP3	12	YTH 63	IR64-NIL1	Sasaki et al. 2017	*qTSN12.1*
	qTSN12.2 -YP4	12	YTH 83	IR64-NIL12	Sasaki et al. 2017	*qTSN12.2*
Seed length (SL)						
	qSL7.1 -YP7	7	YTH 270	IR64-NIL13	Koide et al. 2013	*qSL7.1*
Seed width (SW)						
	qSW2.1 -YP7	2	YTH 270	na	Koide et al. 2013	*qSW2.1*
	qSW6.1 -YP7	6	YTH 270	na	Koide et al. 2013	*qSW6.1*
Filled spikelet number (FSN)						
	qFSN4.1 -YP8	4	YTH 288	IR64-NIL4	Tagle et al. 2016	*qFSN4.1*
100-grain weight (GW)						
	qGW5.1 -YP1	5	YTH 33	IR64-NIL14	unpublished	
	qGW5.2 -YP5	5	YTH 199	IR64-NIL15	unpublished	
	qGW5.3 -YP10	5	YTH 342	IR64-NIL16	unpublished	
Grain length (GRL)						
	qGRL5.1 -YP10	5	YTH 342	IR64-NIL16	unpublished	
Grain width (GRW)						
	qGRW5.1 -YP10	5	YTH 342	IR64-NIL16	unpublished	
Culm length (CL)						
	qCL4.1 -YP8	4	YTH 288	IR64-NIL4	Tagle et al. 2016	*qCL4.1*
Flag leaf length (FLL)						
	qFLL2.1 -YP8	2	YTH 288	IR64-NIL19	Tagle et al. 2016	*qFLL2.1*
	qFLL4.1 -YP8	4	YTH 288	IR64-NIL4	Tagle et al. 2016	*qFLL4.1*
	na	2	YTH 289	na	Farooq et al. 2010	*qFLLnpt-2*
	na	4	YTH 289	na	Farooq et al. 2010	*qFLLnpt-4*
Flag leaf width (FLW)						
	qFLW4.1 -YP8	4	YTH 288	IR64-NIL4	Tagle et al. 2016	*qFLW4.1*
	na	4	YTH 289	na	Farooq et al. 2010	*qFLWnpt-4*
	na	1	YTH 72	na	Farooq et al. 2010	*qFLWnpt-1*
Leaf length (LL)						
	na	1	YTH 197	na	Farooq et al. 2010	*qLLnpt-1*
Leaf width (LW)						
	na	4	YTH 155	na	Farooq et al. 2010	*qLWnpt-4a*
	na	2	YTH 205	na	Farooq et al. 2010	*qLWnpt-2*
	na	4	YTH 318	na	Farooq et al. 2010	*qLWnpt-4b*
Root length (RL)						
	qRL5.1 -YP1	5			Obara et al. 2014	*qRL5.1*-YP1
	qRL5.2 -YP10	5			Obara et al. 2014	*qRL5.2*-YP10
	qRL6.3 -YP10	6			Obara et al. 2014	*qRL6.3*-YP10
	qRL6.4 -YP5	6			Obara et al. 2014	*qRL6.4*-YP5
	qRL7.1 -YP1	7			Obara et al. 2014	*qRL7.1*-YP1

[a] na: not available.

2. Phenotypic and genotypic evaluation

F_2 plants were grown together with the parents in the experimental field at IRRI for phenotypic evaluation as described in the previous section. Genotyping was performed on individual plants of the segregating populations using SSR markers polymorphic between the selected ILs and IR64 as described in the previous section. QTL analysis was performed with version 2.5 of the QTL Cartographer software using the single-marker analysis and composite interval mapping (CIM) approaches (Wang et al. 2011). The LOD score threshold for QTL detection was calculated by a 1000-permutation test. The recombination values between SSR markers were estimated based on the genotypes of the F_2 population using the maximum-likelihood method (Allard 1956), and then converted to centimorgans (cM) using the Kosambi map function (Kosambi 1943).

3. Detection of QTLs

(1) Days to heading

Three QTLs for low DTH and two QTLs for high DTH were identified by single-marker analysis in segregating populations derived from crosses between selected ILs and IR64 (Fujita et al. 2011 and Table 2). Among these five QTLs, one QTL was detected by CIM in each of the four populations: two for low DTH and two for high DTH.

In addition to the five QTLs described above, four QTLs for DTH were also detected (Table 2). A QTL for low DTH was detected on chromosome 6 (*qDTH6.3*-YP10 in YTH339/IR64), and QTLs for high DTH on chromosome 2 (*qDTH2.1*-YP8 in YTH288/IR64, Tagle et al. 2016), chromosome 4 (*qDTH4.1*-YP5 in YTH222/IR64), and chromosome 8 (*qDTH8.2*-YP5 in YTH222/IR64).

Many QTLs for DTH have been mapped on rice chromosomes, and some have been isolated by map-based cloning (Yano et al. 2000, Takahashi et al. 2001, Kojima et al. 2002, Doi et al. 2004, Xue et al. 2008). *qDTH8.1*-YP1 was detected in the same region of chromosome 8 as *Hd5* (Lin et al. 2003). *qDTH6.1*-YP3 corresponded to the location of *Hd3a* and *Hd3b* on chromosome 6 (Kojima et al. 2002, Monna et al. 2002). *qDTH11.1*-YP6 corresponded to *QHd11* and *dth11.1* in the centromere region of chromosome 11 (Li et al. 2003, Septiningsih et al. 2003). The QTL on chromosome 11 was identified in the same region as *hd11* (Yu et al. 2002). *qDTH6.2*-YP7 corresponded to the location of *Hd1* on chromosome 6 (Yano et al. 2000). The location of *qDTH2.1* was similar to that of *Hd7* (Yamamoto et al. 2000). The RFLP marker C560 closest to *Hd7* was identified on the long arm of chromosome 2, and *Hd7* was located between R3393 (29.2 Mb) and R2511 (35.9 Mb). *qDTH2.1*-YP8 was detected between RM5472 (31.5 Mb) to RM240 (32.4 Mb). The location of *Hd7* overlapped with that of *qDTH2.1*, although further study is needed to clarify their relationship.

(2) Total spikelet number per panicle

ILs derived from the five donor varieties contained regions on the long arm of chromosome 4 associated with high TSN (Fujita et al. 2009). To determine the precise location of QTLs for TSN on the long arm of chromosome 4, CIM was conducted in the five populations. A single QTL for high TSN was detected in each segregating population. The QTL (*qTSN4.1*-YP4 in YTH83) was identified within a 1.9-cM interval flanked by RM17470 and RM3534 (location of peak LOD score); it contributed 8.4% of phenotypic variation. *qTSN4.2*-YP5 in YTH191 was identified within a 0.3-cM interval flanked by RM3534 and RM17486; it contributed 15.8% of phenotypic variation. *qTSN4.3*-YP8 in YTH288 was identified within a 3.0-cM interval flanked by RM6480 and RM5503; it contributed 8.0% of phenotypic variation. *qTSN4.4*-YP9 in YTH326 was identified within a 15.2-cM interval flanked by RM3843 and RM1113; it contributed 26.6% of phenotypic variation. *qTSN4.5*-YP11 in YTH155 was identified within a 4.4-cM interval flanked by RM17450 and RM17470; it contributed 38.2% of phenotypic variation. At each of these QTLs, the allele of the donor parent increased TSN.

The locations of these five QTLs were mapped by using F_2 segregating populations. The LOD peaks of these QTLs were located between RM5503 and RM3836 within a 1.44-Mb region. The most precisely mapped QTL (*qTSN4.4*-YP9) was delimited to the 398-kb region between RM3423 and RM17492. Several QTLs for TSN have been reported on the long arm of chromosome 4 (Zhuang et al. 1997, He et al. 2001, Brondani et al. 2002, Lafitte et al. 2002, Hittalmani et al. 2003, Lanceras et al. 2004, Ashikari et al. 2005, Mei et al. 2005, Zou et al. 2005, Li et al. 2006).

Another QTL for TSN was mapped on chromosome 7 (Koide et al. 2013). One of the ILs, YTH270 derived from Hoshiaoba, had a significantly higher TSN than IR64. CIM analysis detected one putative QTL (*qTSN7.1*-YP7) on chromosome 7 between the markers RM1132 and RM505. The positive allele of *qTSN7.1*-YP7 came from YTH270 (and therefore originated from Hoshiaoba), which was consistent with the high TSN of YTH270.

To date, more than 200 QTLs for grain number have been reported in rice, and many have been mapped to specific chromosomes (http://www.gramene.org/). Several genes that control grain number (e.g., *Gn1a*, *DEP1*, *OsSPL14*, *APO1*/*SCM2*, and *Ghd7*) have been cloned (Ashikari et al. 2005, Xue et al. 2008, Huang et al. 2009, Ikeda-Kawakatsu et al. 2009, Jiao et al. 2010, Miura et al. 2010, Ookawa et al. 2010). Although *Ghd7* is located on chromosome 7, its position (9.2 Mb) is far from that of *qTSN7.1*-YP7 (24.0-26.0 Mb), indicating that *qTSN7.1*-YP7 is not *Ghd7*. In the region of *qTSN7.1*-YP7, several QTLs for spikelet number have been reported. Jiang et al. (2004) reported two such QTLs in a doubled-haploid population

derived from a cross between *indica* and *japonica* varieties. Obara et al. (2001) detected two QTLs for spikelet number on chromosome 7 by using ILs derived from a cross between *indica* and *japonica* varieties. One of these QTLs was detected in the same region as *qTSN7.1*-YP7. Mei et al. (2006) detected a QTL (*qSNP-7*) for spikelet number near *qTSN7.1*-YP7 in a population derived from a cross between a *japonica* variety from the U.S. and an *indica* variety from China. Bai et al. (2010) reported a QTL (*qGL7*) that controls grain size and affects the number of spikelets per panicle near *qTSN7.1*-YP7. Although it is unclear whether *qTSN7.1*-YP7 and the reported QTLs are identical, the co-localization of the QTLs for spikelet number per panicle in this chromosomal region confirms the reliability of QTL detection in our study. The high-resolution mapping and cloning of the genes responsible will help to more precisely identify these QTLs.

In the association analysis described in the previous section, the presence of QTLs that increase TSN was predicted on chromosome 12. YTH63 and YTH83, which showed higher TSN than IR64 and had an introgressed segment on chromosome 12, were crossed with IR64 to generate segregating populations. In each population, QTLs named *qTSN12.1*-YP3 and *qTSN12.2*-YP4 for high TSN, whose positive alleles were derived from YP3 and YP4, respectively, were located on the long arm of chromosome 12 as determined by CIM (Table 2) (Sasaki et al. 2017).

(3) Grain size and weight

In the population used to detect *qTSN7.1*-YP7, QTLs for seed size were detected by measuring the seed length (SL), seed width (SW), and seed thickness (ST) of fully filled grains with hulls (Koide et al. 2013). In single-marker analysis, eight markers located on chromosome 7 were significantly associated with SL. RM1132 (located at 24.6 Mb) showed the highest *F* value. In CIM analysis, a LOD peak was observed between RM505 and RM234. The positive allele of this QTL came from IR64. We have provisionally designated the QTL as *qSL7.1*-YP7. Two markers on chromosome 2 and one on chromosome 6 were significantly associated with SW in single-marker analysis. Among the two markers on chromosome 2, RM5631 showed a higher *F* value. We have provisionally designated the QTLs on chromosomes 2 and 6 as *qSW2.1*-YP7 and *qSW6.1*-YP7, respectively. In CIM analysis, a LOD peak was observed between the markers RM3512 and RM5631 on chromosome 2; the positive allele of this QTL came from YTH270.

In the F_2 population derived from the cross between YTH270 and IR64, a QTL for SL (*qSL7.1*-YP7) was detected near *qTSN7.1*-YP7. Grain number and grain size are reportedly controlled by a single gene or QTL with a pleiotropic effect (Song et al. 2007, Bai et al. 2010). Song et al. (2007) showed that a NIL carrying the gene *GW2*, which increases grain length and weight, had a smaller grain number than its recurrent parent. Bai et al. (2010) suggested that *qGL7* had a pleiotropic effect on grain number, length, and weight. Interestingly, *qGL7* has been mapped near *qTSN7.1*-YP7. We also found the co-localization of QTLs controlling TSN and SL on chromosome 7. These results suggest that the higher TSN and lower SL in IR64-NIL13 than in IR64 are controlled by the same QTL with a pleiotropic effect, although it is also possible that the genes for TSN and SL are closely linked. The identification of *qTSN7.1*-YP7 might reveal the molecular mechanisms responsible for its pleiotropic effect on TSN and SL.

YTH33 (derived from YP1), YTH199 (from YP5), and YTH342 (from YP10) showed higher GW than IR64. By analyzing the F_2 populations derived from crosses between IR64 and these ILs, we detected a QTL for GW on chromosome 5 in each population; *qGW5.1*-YP1 from YTH33, *qGW5.2*-YP5 from YTH199, and *qGW5.3*-YP10 from YTH342 (Fujita et al. unpublished). The NPT alleles of all three QTLs increased GW.

We also conducted QTL analysis of grain shape using F_2 populations derived from YTH342/IR64, as YTH342 had the fewest introgressed segments (Fujita et al. unpublished). Grain width (GRW) of YTH342 was higher than that of IR64, whereas grain length (GRL) was lower. CIM revealed QTLs for GRW, *qGRW5*-YP10 and GRL, *qGRL5*-YP10 in the *qGW5.3*-YP10 region of chromosome 5 (Table 2). The NPT allele of *qGRW5*-YP10 increased GRW, whereas the IR64 allele of *qGRL5*-YP10 increased GRL.

Using SSR markers, we detected three QTLs for high GW—*qGW5.1*-YP1, *qGW5.2*-YP5, and *qGW5.3*-YP10—and two for grain size—*qGRL5*-YP10 and *qGRW5*-YP10 (Fujita et al. unpublished, Table 2). Substitution mapping placed *qGW5.1*-YP1 and *qGW5.2*-YP5 at a different location from that of *GS5,* and overlapped with that of *qSW5/GW5* (Shomura et al. 2008, Weng et al. 2008, Li et al. 2011). The precise location of *qGW5.1*-YP10 is difficult to compare with those of *qSW5*, *GW5*, and *GS5* because the limited number of recombinants restricts the resolution of substitution mapping.

qSW5/GW5 increases GW, GRW, and grain thickness (Shomura et al. 2008, Weng et al. 2008). A QTL detected in our study also increased GRW and thickness. *qSW5* was identified in a segregating population derived from a cross between *japonica* Nipponbare and *indica* Kasalath (Shomura et al. 2008). *GW5* was detected in a population derived from a cross between *japonica* Asominori and *indica* IR24 (Weng et al. 2008). Most temperate and tropical *japonica* cultivars have the Nipponbare allele of *qSW5/GW5*, which increases GW. The *japonica* allele of *qSW5* has a 1212-bp deletion in this region. PCR-based marker analysis suggested that the *qSW5* allele in our three ILs is the same as the Nipponbare allele (Fujita et al. unpub-

lished). Therefore, the detected QTLs may be the same as *qSW5/GW5*.

(4) Leaf size

Using several ILs with unique LL and LW, QTL analysis was conducted for leaf size traits (Farooq et al. 2010). Eight QTLs (mapped on three chromosomes) were identified for the leaf size traits in six F_2 populations. Two QTLs for flag LL (FLL)—*qFLLnpt-2* and *qFLLnpt-4*—in YTH289/IR64 were identified on chromosomes 2 and 4, respectively. Two QTLs for flag LW (FLW) were identified: *qFLWnpt-4* in YTH289/IR64 near RM17483 on chromosome 4 and *qFLWnpt-1* in YTH72/IR64 near RM3252 on chromosome 1. A QTL for LL (*qLLnpt-1*) in YTH197/IR64 was identified near RM3709 on chromosome 1. Three QTLs for LW were identified: *qLWnpt-2* in YTH205/IR64 near RM7451 on chromosome 2, *qLWnpt-4a* in YTH155/IR64 near RM7208 on chromosome 4, and *qLWnpt-4b* in YTH318/IR64 near RM6909 on chromosome 4.

(5) Root development

A greater variation in maximum root length (RL) was found in three of the sib IL groups (Obara et al. 2014). Five QTLs were detected by single-marker analyses: two on chromosomes 5 and 6 and one on chromosome 7 (Table 2). The most effective QTL (*qRL6.4*-YP5) was derived from YP5 and located on the long arm of chromosome 6. The effect of this QTL on root elongation was confirmed by evaluating a NIL for the QTL.

(6) Other traits related to the NPT

YTH288 had several agronomically valuable characteristics such as large panicles, few unproductive tillers, and large leaves inherited from NPT varieties. To identify the genetic basis of these traits, 167 F_2 plants derived from a cross between IR64 and YTH288 were used for QTL analysis (Tagle et al. 2016). Four putative QTLs were detected on chromosome 4 (for CL, FLL, FLW, and filled spikelet number per panicle [FSN]) and two on chromosome 2 (for DTH and FLL). The YP8 alleles had positive effects, except for the QTL for FLL on chromosome 2.

Using SSR markers in the introgressed segments, we detected four QTL (*qCL4.1*-YP8, *qFLL4.1*-YP8, *qFLW4.1*-YP8, and *qFSN4.1*-YP8; Table 2) on the long arm of chromosome 4 between RM6480 and RM3843. The peak LODs fell between RM17470 and RM3534 for *qCL4.1*-YP8, between RM6909 and RM3843 for *qFLL4.1*-YP8, between RM3534 and RM17483 for *qFLW4.1*-YP8, and between RM6480 and RM5503 for *qFSN4.1*-YP8. *qDTH2.1*-YP8 and *qFLL2.1*-YP8 were both located between RM5472 and RM240 on the long arm of chromosome 2, which explained 9% to 28% of the phenotypic variation, respectively. The YP8 alleles had positive effects, except for *qFLL2.1*-YP8.

QTLs for agronomic traits in rice have been identified on all chromosomes. Yonemaru et al. (2010) reported QTL clusters associated with multiple morphological traits on chromosomes 1, 3, 4, 6, and 9. QTL clusters for morphological traits such as leaf size, spikelet number, PN, and root volume are located on the long arm of chromosome 4 (http://qtaro.abr.affrc.go.jp/). In our study, four QTL for CL, FLL, FLW, and FSN were co-localized on the long arm of chromosome 4 (Table 2). QTLs for various agronomic traits were previously identified in this region: for GW and plant height (Yagi et al. 2001); FLL and FLW (Mei et al. 2005); FLL, FLW, FLA, PL, PN, and grain yield (Zou et al. 2005, Yue et al. 2006); FLW and PL (Kobayashi et al. 2006); and FLW and root volume per tiller (Ding et al. 2011). The co-localization of QTLs for various traits suggests either a gene cluster or the pleiotropic effect of a gene underlying these QTLs. Thus, the QTLs derived from NPT varieties detected on the long arm of chromosome 4 need fine mapping.

QTLs for large panicles and broad leaves may contribute to the higher yield of YTH288 than IR64 (Tagle et al. 2016). We detected QTLs for leaf size (*qFLL4.1*-YP8 and *qFLW4.1*-YP8) on the long arm of chromosome 4, where Yue et al. (2006) detected *QFll4*, *QFlw4*, and *QFla4*. *qFLL4.1*-YP8 was located between RM3843 (32.2 Mb) and RM6909 (32.6 Mb), and *qFLW4.1*-YP8 between RM3534 and RM17483 (both at 31.7 Mb). Similarly, *QFll4*, *QFlw4*, and *QFla4* were detected between RM255 (31.5 Mb) and RM349 (33.2 Mb). *qFSN4.1*-YP8 associated with panicle structure was also located on the long arm of chromosome 4 between RM6480 (30.4 Mb) and RM5503 (30.9 Mb). QTLs affecting panicle structure were previously reported in a similar region; *qNOS4-3* near RG214 (32.3 Mb), and *qSN-4a* between RM317 (29.7 Mb) and RM255 (31.5 Mb) (Hittalmani et al. 2003, Zou et al. 2005). A QTL for FLW, spikelet number, and root volume has been mapped within 38 kb on the long arm of chromosome 4 (Ding et al. 2011), and we identified a gene that increases TSN in this region (Fujita et al. 2013). More detailed analysis is needed to determine whether the QTLs detected in our studies are the same as those previously reported.

Development and characterization of near-isogenic lines with the genetic background of IR64

To evaluate the effects of detected QTLs, we developed and characterized a total of 20 NILs for DTH, TSN, and GW with the genetic background of IR64.

1. Selection scheme and evaluation

From among the F_2 populations (equivalent to BC_4F_2) used for QTL mapping described in the previous section, plants having the desired introgressions were selected (Fig. 3). The plant with the fewest and smallest introgressed segments in non-target chromosomal regions was chosen from each population for self-pollination to develop a fixed line.

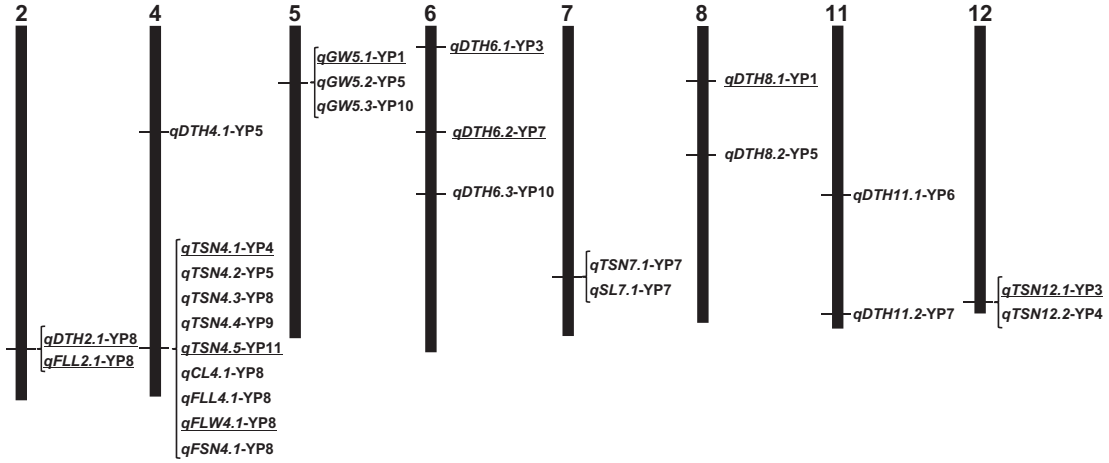

Fig. 3. Position of QTLs for unique agronomic traits in developed IR64-NILs
The underlined QTLs are identified using 334 IR64 ILs.

Table 3. Developed IR64-NILs for unique agronomic traits

Entry no.	QTL	Marker interval[a]			Trait at IRRI
IR64-NIL1	*qTSN12.1* -YP3				high spikelet number
IR64-NIL2	*qTSN4.1* -YP4	RM17470	–	RM3534	high spikelet number
IR64-NIL3	*qTSN4.2* -YP5	RM3534	–	RM17486	high spikelet number
IR64-NIL4	*qTSN4.3* -YP8	RM6480	–	RM5503	high spikelet number
	qCL4.1 -YP8	RM17470	–	RM3534	long culm
	qFLL4.1 -YP8	RM6909	–	RM3843	long flag leaf
	qFLW4.1 -YP8	RM3534	–	RM17483	wide flag leaf
	qFSN4.1 -YP8	RM6480	–	RM5503	high fertile spikelet number
IR64-NIL5	*qTSN4.4* -YP9	RM3843	–	RM1113	high spikelet number
IR64-NIL6	*qTSN4.5* -YP11	RM17450	–	RM17470	high spikelet number
IR64-NIL7	*qDTH8.1* -YP1	RM5556	–	RM6838	early heading
IR64-NIL8	*qDTH6.1* -YP3	RM588	–	RM587	late heading
IR64-NIL9	*qDTH11.1* -YP6	RM3428	–	RM5582	late heading
IR64-NIL10	*qDTH6.2* -YP7	RM6302	–	RM7311	early heading
IR64-NIL11	*qDTH11.2* -YP7	RM224[b]			early heading
IR64-NIL12	*qTSN12.2* -YP4				high spikelet number
IR64-NIL13	*qTSN7.1* -YP7	RM1132	–	RM50	high spikelet number
	qSL7.1 -YP7	RM1132	–	RM50	short seed
IR64-NIL14	*qGW5.1* -YP1				heavy grain
IR64-NIL15	*qGW5.2* -YP5				heavy grain
IR64-NIL16	*qGW5.3* -YP10				heavy grain
IR64-NIL17	*qDTH4.1* -YP5				late heading
IR64-NIL18	*qDTH8.2* -YP5				late heading
IR64-NIL19	*qDTH2.1* -YP8	RM5472	–	RM240	late heading
	qFLL2.1 -YP8	RM5472	–	RM240	short flag leaf
IR64-NIL20	*qDTH6.3* -YP10				early heading

[a] The closest markers are underlined.
[b] Single-marker analysis.

Several developed NILs had introgressed segments other than the target chromosome region, but these regions were not associated with the target traits, supporting the presence of a single QTL for each target trait in each NIL. Developed NILs were characterized in the experimental field at IRRI as described in the previous section.

2. NILs for agronomic traits

(1) Days to heading

Nine NILs for DTH were developed (Table 3). IR64-NIL7 for *qDTH8.1*-YP1, derived from YTH12, had introgressed segments from YP1 on chromosome 8 (Fujita et al. 2011) (Fig. 3). IR64-NIL8 for *qDTH6.1*-YP3, derived from YTH74, had an introgressed segment from YP3 on the short

arm of chromosome 6. IR64-NIL9 for *qDTH11.1*-YP6, derived from YTH252, had introgressed segments from YP6 on chromosome 11. IR64-NIL10 for *qDTH6.2*-YP7 and IR64-NIL11 for *qDTH11.2*-YP7, derived from YTH277, had introgressed segments from YP7 on the long arm of chromosome 6 and 11, respectively. In addition to these five NILs reported previously (Fujita et al. 2011), four NILs (IR64-NIL17 for *qDTH4.1*-YP5, IR64-NIL18 for *qDTH8.2*-YP5, IR64-NIL19 for *qDTH2.1*-YP8, and IR64-NIL20 for *qDTH6.3*-YP10) were developed through the same scheme. IR64-NIL19 also carried *qFLL2.1*-YP8 for a long flag leaf located in the same region of chromosome 2 as *qDTH2.1*-YP8 (Tagle et al. 2016).

The DTH of IR64-NIL7 and IR64-NIL10 was 5 days shorter than that of IR64, whereas those of IR64-NIL8 and IR64-NIL9 were 7-10 days longer. Although the NILs were morphologically similar to IR64, significant differences were observed in several agronomic traits (Fujita et al. 2011). IR64-NIL7 had low LW and high PN compared with IR64. CL, PL, LL, and LW were higher in IR64-NIL8 than in IR64. IR64-NIL9 had higher CL and lower LW than IR64. Agronomic traits other than DTH in IR64-NIL10 were not significantly different from those of IR64.

These NILs were developed through marker-assisted selection and are probably suitable for tropical conditions because IR64 is widely cultivated in tropical areas. Several agronomic characteristics (CL, PL, LL, LW, and PN) of IR64-NIL7, IR64-NIL8, and IR64-NIL9 differed from those of IR64, although most of their genomes originated from IR64. These results suggested that the change in growth duration influenced agronomic traits. It is also possible that chromosomal segments that affect CL, PL, LL, LW, and PN remained in the NILs. Further molecular genetic studies using NILs may reveal the cause of the differences in agronomic traits.

(2) Total spikelet number per panicle

Eight NILs for TSN (five for *qTSN4*, two for *qTSN12*, and one for *qTSN7*) were developed. All five NILs (IR64-NIL2, IR64-NIL3, IR64-NIL4, IR64-NIL5, and IR64-NIL6) for *qTSN4* were developed using different donor parents (Fujita et al. 2012) (Table 3, Fig. 3). IR64-NIL4 also carried four QTLs for unique agronomic traits inherited from the NPT parent: *qCL4.1*-YP8 for CL, *qFLL4.1*-YP8 for FLL, *qFLW4.1*-YP8 for FLW-YP8, and *qFSN4.1*-YP8 for FSN (Tagle et al. 2016).

IR64-NIL13 for *qTSN7.1*-YP7 was developed using YTH270 as a parent (Koide et al. 2013). This NIL also carried *qSL7.1*-YP7 for short grain located in the same region of chromosome 7 as *qTSN7.1*-YP7. IR64-NIL1 for *qTSN12.1*-YP3 and IR64-NIL12 for *qTSN12.2*-YP4 had high TSN; both QTLs were located on chromosome 12 (Sasaki et al. 2017).

The five NILs for *qTSN4*, which carried introgressed segments on chromosome 4 derived from different donor parents, differed significantly from IR64 in several agronomic traits (Fujita et al. 2012). All five NILs had significantly higher CL, FLL, LW, FLW, and FSN than IR64. These NILs also had greater PL than IR64, although the difference was not significant for IR64-NIL2. The LL of these NILs was also greater than that of IR64; the difference was not significant for IR64-NIL3 and IR64-NIL4. The 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. IR64-NIL4, which also carried *qCL4.1*-YP8, *qFLL4.1*-YP8, *qFLW4.1*-YP8, and *qFSN4.1*-YP8 on chromosome 4, had significantly higher CL, FLL, FLW, and TSN than those of IR64 (Tagle et al. 2016).

The five NILs for *qTSN4* showed differences in panicle architecture such as the number of branches and spikelet number on each branch. The high TSN of the five NILs was mainly attributed to the increased number of secondary branches. The differences in panicle architecture of the NILs might be explained by differences in the genes (alleles) underlying the QTLs detected in the five crosses.

IR64-NIL13 for *qTSN7.1*-YP7 had a significantly higher TSN, but a significantly lower SL than those of IR64 (Koide et al. 2013). In the field experiment conducted at IRRI during the DS of 2012, GW was significantly lower in IR64-NIL13 than in IR64. No significant differences between IR64-NIL13 and IR64 were detected for the other nine traits.

(3) Grain weight and size

We developed three NILs for GW from the following crosses: IR64-NIL14 for *qGW5.1*-YP1 from YTH33/IR64, IR64-NIL15 for *qGW5.2*-YP5 from YTH199/IR64, and IR64-NIL16 for *qGW5.3*-YP10 from YTH342/IR64 (Fujita et al. unpublished) (Table 3, Fig. 3). We evaluated the GRL, GRW, and grain thickness of the three NILs and IR64 in the IRRI field in the 2010 DS.

The 100-GWs of IR64-NIL14 (with an introgressed segment from YP1 on chromosome 5), IR64-NIL15 (from YP5), and IR64-NIL16 (from YP10) were 0.2-0.4 g higher than that of IR64 (Fujita et al. unpublished). GRW and grain thickness of the three NILs were significantly higher than those of IR64, but GRL was not significantly different.

These NILs for *qGW5* had heavier, wider, and thicker grains than IR64, whereas other traits were similar to those of IR64 (Fujita et al. unpublished). These results suggest that these QTLs will be useful for improving yield potential by increasing grain size and weight.

Conclusions

We genetically dissected the agronomic traits of rice by developing ILs produced by crossing 10 donor parents

with the elite variety IR64. We detected QTLs for agronomic traits by a genetic analysis of hybrid populations, and developed 20 NILs for unique agronomic traits with the genetic background of IR64. These lines are useful in breeding and studies aimed at increasing rice yield.

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